Promoters : Prof. dr. ir. Koen Dewettinck

Laboratory of Food Technology and Engineering
Department of Food Technology, Safety, and Health
Faculty of Bioscience Engineering
Ghent University

dr. Tom Rimaux

Vandemoortele R&D Center
Prins Albertlaan 79
Izegem, Belgium

Dean : Prof. dr. ir. Marc Van Meirvenne

Rector : Prof. dr. ir. Rik Van de Walle

GHENT UNIVERSITY

FACULTY OF BIOSCIENCE ENGINEERING

Mohd Dona Bin Sintang

SYNERGISTIC COMBINATIONS OF STRUCTURANTS FOR EDIBLE LIQUID-OIL SOFT MATTER SYSTEMS

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"*It always seems impossible until it's done*"

Nelson Mandela

Mohd Dona Bin Sintang

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List of abbreviations and symbols

12-hydroxystearic acid	12-HSA
Alpha	α
Angstrom	Å
Beta	β
Complex modulus	|G*|
Degree	°
Gamma	γ
Micrometer	µm
Phase angle/delta	δ
Prime	'
Theta	Θ
Wavelength	λ
Colloidal silicium dioxide	CSD
Coronary heart disease	CHD
Confocal laser scanning microscopy	CLSM
Degree of substitution	DoS
Differential scanning calorimetry	DSC
Distilled monoglycerides from palm oil	DM
Ethylcellulose	EC
Fatty acids	FAs
Fatty alcohols	FALs
Fourier transform infrared spectroscopy	FTIR
Fully hydrogenated rapeseed oil	FHRO
Storage modulus	G'
Loss modulus	G"
Gelatin	GTA
Crystallization enthalpy	H_{cry}
High internal phase emulsion	HIPE
Hydroxypropyl methylcellulose	HPMC
Linear Viscoelastic region	LVR
Loss modulus	G"

Low molecular weight gelators	LMOGs
Melting enthalpy	H_{mp}
Methylcellulose	MC
Monoglycerides	MGs
Nanometer	nm
Non-communicable diseases	NCDs
Phytosterols	PSs
Polarized light microscopy	PLM
Self-assembly fibrillary networks	SAFiNs
Small angle X-ray diffraction	SAXD
Stearic acid	SA
Scanning electron microscopy	SEM
Sucrose esters	SEs
Saturated fatty acid	SFA
Lecithin	SFL
Stearyl alcohol	SO
Triacylglycerides	TAGs
Peak crystallization temperature	T_{cry}
Trans-fatty acid	tFA
Gelling temperature	T_{gel}
Peak melting temperature	T_{mp}
Offset melting temperature	T_{off}
Onset crystallization temperature	T_{on}
Ultra-Small angle X-ray diffraction	USAXD
Wax esters	WE
Wide angle X-ray diffraction	WAXD
X-ray diffraction	XRD

Summary

In the last decade, oleogelation has emerged as an alternative for conventional oil structuring, which relies on saturated and *trans*- fat crystallization. In oleogelation, liquid oil is transformed to soft, solid-like systems using specific structurants. However, only a limited number of edible and GRAS structurants are available and none of the available structurants can imitate the functionality and organoleptic properties of crystalline fat. Thus, more potential can be found in the possible synergistic interactions when different structurants are combined. Therefore, the objective of this PhD research was to find synergies in bi-component systems, which have promising rheological properties compared to their corresponding mono-component systems. Moreover, the synergies among structurants are interesting because they allow the reduction of the total amount of used structurants as a result of their tunable properties.

Introduction to the concept of oleogelation and its applications are presented in **Chapter 1**. Oleogels are categorized in crystalline based systems, self-assembly fibrillar networks (SAFiNs), polymer based structuring, particle based systems, and indirect approaches. However, a structurant can exist as a single (mono-component) or bi-component system. Oleogels have been used widely as a structuring and delivery agent in pharmaceutical and cosmetic products, and recently, been applied as a co-structurant in lipid-based food products. Based on the research on the application of oleogels in pharmaceutical, food, and cosmetic products, it is clear that a specific oleogel system has to be developed. This is because some oleogels lose their functionality from the interaction with other components present in real systems which are naturally complex. Therefore, investigating new oleogels raise the opportunity for the application of oleogels in complex systems. The approaches toward discovery of new oleogels are discussed and outlined in the perspectives section of **Chapter 1**.

In this study, similar characterization techniques were employed in **Chapter 2**, **3**, and **4**. To identify a possible interaction, several combination ratios were first thermally, rheologically, and morphologically characterized. The ratios at which synergistic interactions occurred were further explored to investigate the effect of the combination, mainly on the rheological properties. This characterization was performed using differential scanning calorimetry (DSC), polarized light microscopy (PLM), cryo-

Scanning electorn microscopy (SEM), confocal laser scanning microscopy (CLSM), and X-ray diffraction (XRD) measurement.

Chapter 2 focuses on finding an alternative technique to modify the structuring properties of a structurant based on triacylglycerides (fully hydrogenated rapeseed oil (FHRO)). To modify its structuring properties, the FHRO was transformed into fat capsules which acted as a carrier for hydrophilic polymers. Hydrophilic polymers, such as methylcellulose (MC) and gelatin (GTA), are known to form a gel in water but not in liquid oil. Therefore, a new approach based on the existing emulsion-templated oleogelation technique was introduced. In this approach, MC and GTA acted as a stabilizer of oil-in-water emulsions. During emulsification, MC or GTA adsorbed at the interface and formed a polymer protective layer, coating the crystallized FHRO upon cooling. Subsequently, the emulsion droplets were separated from the continuous water phase through creaming. The droplets were dried to produce polymer coated fat crystals (fat capsules). These fat capsules were characterized to understand their properties (thermal, diffraction/polymorphism, and morphology). The oleogels were then prepared by adding the fat capsules into sunflower oil at a concentration of 12 %wt, followed by a heating procedure. At high temperatures, the fat capsules were melted and released the hydrophilic polymers into sunflower oil. This process allowed simultaneous structuring of FHRO and the hydrophilic polymer. The oleogels prepared from fat capsules (MC-based) exhibited superior oil structuring properties and exhibited promising rheological properties, which was attributed to the presence of both the hydrophilic polymer and the FHRO. The composite oleogels were composed of both crystalline and polymer networks that synergistically structured the sunflower oil. The formation of composite oleogels was confirmed by cryo-SEM and CLSM.

Chapter 3 describes the oil structuring properties of a bi-component system consisting of monoglycerides (MGs) and phytosterols (PSs). The ratios at which synergies occurred in an oleogel, were investigated by characterizing the mixing behavior of both components. The oleogels were then prepared and characterized to understand the oil structuring properties. The 9:1 and 8:2 (MGs:PSs) bi-component oleogels exhibited promising rheological properties with a higher complex modulus than the MGs mono-component-oleogel. The improvement in the oil structuring properties was explained by the formation of a mixed crystal system. This hypothesis was confirmed using

microscopy and diffraction analysis. Microscopic studies revealed that the bi-component oleogels showed an improved spatial distribution than the mono-component oleogel. Moreover, the bi-component oleogels displayed changes in the crystallization, polymorphic transition, diffraction, and morphology compared to the mono-component systems. These changes were the result of interaction between MGs and PSs, which are known to form complexes.

Self-assembled surfactants are known to organize into different organizational arrangements, which are capable to immobilize a liquid phase. **Chapter 4** presents the structuring ability of sucrose esters in combination with lecithins in sunflower oil. It was hypothesized that the self-assembly of sucrose esters (SEs) in sunflower oil, could be modified with sunflower lecithin (SFL) as co-surfactant. The interaction between SEs and SFL was manifested by a change in the shape of the crystallization peak in bi-component oleogels. The crystallization peak became sharper starting from the ratio of 7:3 SEs:SFL and below. Interestingly, the 7:3 SEs:SFL oleogel exhibited promising rheological properties, higher in complex modulus than the mono-component SEs oleogel. The promising rheological properties of bi-component oleogels was correlated to the modulation in the self-assembly of SEs. This was confirmed with electron microscopy and XRD. The solely SEs mono-component oleogel exhibited globular structures, which were absent in the bi-component oleogels. Interestingly, the bi-component oleogels (7:3 and 6:4 of SEs:SFL) displayed different small angle X-ray diffraction (SAXD) patterns compared to SEs mono-component oleogels. Therefore, it was hypothesized that the bi-component oleogels organized into inverse cylindrical hexagonal structure (tubular micelles), leading to improved rheological properties.

Although a considerable series of analyses were performed in each chapter, the exact molecular organization remains uncertain since the concentration of the studied structurants was too low (maximum 12 wt%) to obtain reliable results with powder-XRD. Therefore, in **Chapter 5,** the results of synchrotron radiation XRD were presented to elucidate the molecular organization of selected oleogels researched in each chapter. From Chapter 2, the FHRO-based and fat capsules of MC-based oleogels were selected for analysis. The SAXD and WAXD results exhibited no substantial difference in the diffraction patterns (crystallization and melting) between the FHRO-based and MC-based oleogels. These outcomes supported the results

obtained in Chapter 2. From Chapter 3, the oleogels prepared from 10:0, 6:0, 6:4, and 0:10 MGs:PSs were selected for synchrotron X-ray analysis. The SAXD results showed that the first peak of 6:0 appeared earlier than the 6:4 MGs:PSs oleogels during cooling, and the WAXD peak at 4.11Å observed in the 6:0 was absent in the 6:4 MGs:PSs oleogels. These results strongly supported the hypothesis of complex formation between MGs and PSs which influences the crystallization and transition of MGs. The bi-component oleogels from SEs and SFL mixture of Chapter 4, revealed interesting diffraction SAXD patterns. The SAXD pattern of the 10:0 SEs:SFL oleogel exhibited multiple peaks which could be attributed to lamellar and probably hexagonal configurations. However, only two observable peaks appeared in the 6:4 SEs:SFL oleogel, which was hypothesized to organize into an inverse cylindrical hexagonal structure. Interestingly, no diffraction peaks were observed in the WAXD region for all studied SEs:SFL oleogels.

In general, this study expands the available structurants that can be used as alternatives to a traditional saturated TAG-based hardstock. In **Chapter 6**, it was concluded that the studied structurants belonged to different categories of oleogels: crystalline based, self-assembly fibrillar networks and indirect structuring. Moreover, it could also be concluded that the structuring features did not rely solely on lamellae organization, as in conventional oil structuring. This study found that the inverse cylindrical hexagonal structure is also capable of structuring sunflower oil. Additionally, it was demonstrated that in certain circumstances, specific techniques or approaches can allow the incorporation of incompatible structurants (less soluble) into edible liquid oil. This was demonstrated by the incorporation of the hydrophilic polymers and mixing of sucrose esters into sunflower oil. Ultimately, this study provides the basis for other researchers to explore fundamental and application aspects of the studied oleogels.

Samenvatting

Gedurende het laatste decennium is olie-gelering naar voor geschoven als een alternatief voor conventionele olie-structurering welke gebaseerd is op verzadigd en trans-vet kristallisatie. Bij het geleren van olie wordt vloeibare olie omgezet tot zachte, vaste systemen door gebruik te maken van specifieke structuranten. Echter, slechts een beperkt aantal eetbare en GRAS structuranten zijn beschikbaar en geen daarvan blijkt geschikt te zijn om de functionaliteit en organoleptische eigenschappen van kristallijn vet optimaal na te bootsen. Daaruit volgt dat mogelijke synergistische interacties waarin verschillende structuranten gecombineerd worden meer potentieel kunnen bevatten. Bijgevolg was het doel van dit doctoraatsonderzoek om synergistische relaties te vinden in bi-component systemen die veelbelovende reologische eigenschappen bezitten in vergelijking met hun overeenkomstige mono-component systemen. Bovendien is de studie van synergiën tussen structuranten veelbelovend aangezien deze een daling van de totale hoeveelheid gebruikte structuranten met zich meebrengen door hun regelbare eigenschappen.

Hoofdstuk 1 beschrijft het concept 'olie-gelering' en de toepassingen daarvan. Oleogels worden onderverdeeld in kristallijn-gebaseerde systemen, zelf-assembleerbare fibrillaire netwerken, polymeer-gebaseerde structurering, deeltjes-gebaseerde systemen en indirecte benaderingen. Een structurant kan voorkomen als een enkele (mono-component) of een bi-component systeem. Oleogels worden wijdverspreid gebruikt als structurering- en leveringsmiddel in farmaceutische en cosmetica producten. Verder werden deze recentelijk ook toegepast als co-structurant in vet-gebaseerde voedingsproducten. Bepaalde oleogels verliezen hun functionaliteit door de interactie met andere componenten die aanwezig zijn in reële systemen die van nature meer complex zijn, waardoor de ontwikkeling van een specifiek oleogel-systeem voor deze toepassingen noodzakelijk is. Deze verscheidene invalshoeken voor het ontdekken van nieuwe oleogels werden besproken en geschetst in de perspectieven sectie van **Hoofdstuk 1**.

In **Hoofdstuk 2**, **3** en **4** werden gelijkaardige karakterisatie-technieken gebruikt. Er werden mogelijke interacties geïdentificeerd door verschillende ratio's waarin de structuranten werden gecombineerd thermisch, reologisch en morfologisch te karakteriseren. De verhoudingen in dewelke synergistische interacties plaatsvonden

werden verder onderzocht om op die manier het effect van de combinatie op de reologische eigenschappen verder uit te klaren. Deze karakterisatie werd uitgevoerd met behulp van differentiële scanning calorimetrie (DSC), gepolariseerd licht microscopie (PLM), cryo-scanning elektronmicroscopie (cryo-SEM), confocale laser scanning microscopie (CLSM) en X-straal diffractie (XRD) metingen.

Hoofdstuk 2 legt de focus op het vinden van een alternatieve techniek om de structurele eigenschappen van structurant-gebaseerde triglyceriden (volledig gehydrogeneerde raapzaad (FHRO)) te wijzigen. Om de structurerende eigenschappen te wijzigen, werd FHRO omgezet in vetcapsules die functioneerden als drager voor hydrofiele polymeren. Bij hydrofiele polymeren, zoals methylcellulose (MC) en gelatine (GTA), werd reeds gevonden dat ze een gel in water vormen, maar niet in vloeibare olie. Daarom werd een nieuwe aanpak geïntroduceerd op basis van de bestaande emulsie-*templated* olie-geleringstechniek. Bij het toepassen van deze techniek, functioneerden MC en GTA als stabilisatoren van de olie-in-water emulsies. Tijdens emulgering adsorbeerde MC of GTA aan de interface en vormde een beschermende polymeerlaag die de gekristalliseerde FHRO bedekte tijdens afkoeling. Vervolgens werden de emulsie druppels gescheiden van de continue waterfase door oproming. De druppels werden gedroogd om polymeer bedekte vetkristallen (vetcapsules) te verkrijgen. Deze vetcapsules werden vervolgens gekarakteriseerd om hun eigenschappen (thermische, diffractie en morfologische) te begrijpen. De oleogels werden daarna bereid door de vetcapsules toe te voegen aan zonnebloemolie tot een concentratie van 12 %massa werd bekomen, gevolgd door een verwarmingsprocedure. Bij hoge temperaturen smolten de vetcapsules en kwamen de hydrofiele polymeren in de zonnebloemolie vrij. Dit proces liet gelijktijdige structurering van FHRO en het hydrofiele polymeer toe. De oleogels, bereid uit de vetcapsules (MC-gebaseerde), vertoonden superieure olie-structurerende en veelbelovende reologische eigenschappen die werden toegeschreven aan de aanwezigheid van zowel het hydrofiele polymeer als de FHRO. De samengestelde oleogels waren opgebouwd uit zowel kristallijne als polymeernetwerken die de zonnebloemolie synergistisch structureerden. De vorming van dit soort oleogels werd bevestigd door cryo-SEM en CLSM.

Hoofdstuk 3 beschrijft de olie-structurerende eigenschappen van een bi-component systeem bestaande uit monoglyceriden (MG) en fytosterolen (FS). De verhoudingen

waarbij synergiën in een oleogel bekomen werden, werden vervolgens onderzocht door het menggedrag van beide componenten te karakteriseren. De oleogels werden daarna bereid en gekarakteriseerd om de olie-structurerende eigenschappen verder te begrijpen. De 9:1 en 8:2 (MG:FS) bi-component oleogels vertoonden veelbelovende reologische eigenschappen met een hogere complexe modulus dan de MG mono-component oleogels. De verbetering van de olie-structurerende eigenschappen werd verklaard door de vorming van een gemengd kristal systeem. Deze hypothese werd bevestigd met behulp van microscopische en diffractie analyse. Microscopische studies toonden aan dat de bi-component-oleogels een betere ruimtelijke verdeling hadden dan de mono-component oleogels. Bovendien toonden de bi-component oleogels veranderingen in kristallisatie, polymorfe overgang, diffractie en morfologie vergeleken met de mono-component oleogels. Deze veranderingen konden worden toegeschreven aan de interactie tussen MG en FS welke gekend zijn complexen te vormen.

Zelf-geassembleerde oppervlakte-actieve stoffen zijn gekend om zich te organiseren in verschillende structuren die in staat zijn om een vloeibare fase te immobiliseren. **Hoofdstuk 4** geeft het structurerend vermogen weer van sucrose esters gecombineerd met lecithinen in zonnebloemolie. Er werd verondersteld dat de zelf-assemblage van sucrose esters (SE) in zonnebloemolie zou kunnen worden gewijzigd door het toevoegen van zonnebloemlecithine (ZBL) als co-surfactant. De interactie tussen SE en ZBL werd aangetoond door een verandering in de vorm van de kristallisatiepiek bij de bi-component oleogels. De kristallisatiepiek werd scherper startende vanaf de verhouding 7:3 SE:ZBL en alle verhoudingen daaronder. Interessant was dat de 7:3 SE:ZBL oleogel waardevolle reologische eigenschappen vertoonde, met name een hogere complexe modulus dan de mono-component SE oleogel. De interessante reologische eigenschappen van bi-component oleogels waren verder gecorreleerd met de aanpassing in de zelf-assemblage van de SE. Dit werd bevestigd met elektronenmicroscopie (cryo-SEM) en XRD. De SE mono-component oleogels vertoonden bolvormige structuren welke afwezig waren in de bi-component oleogels. De bi-component oleogels (7:3 en 6:4 SE:ZBL) vertoonden verder verschillende *small angle* X-straal diffractie (SAXD) patronen. Daardoor werd verondersteld dat de bi-component oleogels georganiseerd waren in inverse

cilindrische zeshoekige structuren (buisvormige micellen), wat leidde tot verbeterde reologische eigenschappen.

Hoewel in elk hoofdstuk een aanzienlijke reeks analyses werd uitgevoerd, blijft de exacte moleculaire organisatie onzeker, aangezien de concentratie van de bestudeerde structuranten te laag was (maximaal 12 %massa) om betrouwbare resultaten te verkrijgen met behulp van poeder-X-stralen diffractie. In **hoofdstuk 5** werden daarom synchrotron straling XRD resultaten beschreven om de moleculaire organisatie van oleogels, onderzocht in elk hoofdstuk, te verklaren. Uit hoofdstuk 2 werden de FHRO-gebaseerde en vetcapsules van de MC-gebaseerde oleogels geselecteerd voor analyse. De *SAXD*- en *WAXD*-resultaten gaven geen significant verschil in diffractiepatronen (zowel tijdens kristallisering en smelting) tussen de FHRO-gebaseerde en MC-gebaseerde oleogels. Deze resultaten ondersteunden de resultaten die in hoofdstuk 2 verkregen werden. Uit hoofdstuk 3 werden de 10:0, 6:0, 6:4 en 0:10 DM:FS oleogels geselecteerd voor synchrotron analyse. De *SAXD*-resultaten toonden aan dat de eerste piek van 6:0 eerder verscheen dan de 6:4 DM:FS oleogels tijdens de koeling. Verder werd een *WAXD*-piek bij 4.11Å waargenomen in de 6:0 oleogel welke afwezig was in de 6:4 DM:FS oleogels. Deze resultaten vormden een sterke evidentie voor de hypothese van complexvorming tussen DM en FS, welke de kristallisatie en overgang van DM beïnvloedt. De bi-component oleogels van SE en ZBL-mengsels vanuit hoofdstuk 4 onthulden interessante diffractie-*SAXD*-patronen. Het *SAXD* patroon van de 10:0 SE:ZBL oleogel vertoonde meerdere pieken die te wijten waren aan lamellaire en waarschijnlijk zeshoekige configuraties. Echter verschenen er slechts twee waarneembare pieken in de 6:4 SE:ZBL oleogel, welke werd verondersteld om te organiseren in een inverse cilindrische zeshoekige structuur. Verder was het interessant dat er geen diffractietoppen in het *WAXD*-gebied werden waargenomen voor alle bestudeerde SE:ZBL oleogels.

In het algemeen biedt deze studie een waardevolle bijdrage aan de beschikbare structuranten, aangezien zij kunnen gebruikt worden als alternatieven voor een traditionele verzadigde triglyceriden-gebaseerde hardstock. In **hoofdstuk 6** werd geconcludeerd dat de bestudeerde structuranten behoorden tot de verschillende categorieën oleogels: kristallijne, zelf-assemblage fibrillaire netwerken en indirecte structurering. Bovendien kan besloten worden dat de structurele kenmerken niet enkel gebaseerd zijn op lamellenorganisaties zoals bij conventionele oliestructuren het geval

is. Uit deze studie bleek dat de inverse cilindrische hexagonale structuur ook in staat is om zonnebloemolie te structureren. Bovendien werd aangetoond dat in bepaalde omstandigheden specifieke technieken of benaderingen de opname van incompatibele structuranten (minder oplosbaar) in eetbare vloeibare olie toelaten. Dit werd aangetoond door de opname van hydrofiele polymeren en het mengen van sucrose esters in zonnebloemolie. Uiteindelijk biedt dit werk de basis voor andere wetenschappers om fundamentele en toepassingsgerichte aspecten van de bestudeerde oleogels te onderzoeken.

Outline of the research

This doctoral research is conducted within the framework collaboration with Vandemoortele BVBA (Belgium), through Vandemoortele Centre Lipid Science and Technology under the Laboratory of Food Technology and Engineering, Ghent University.

Driven by the need to produce healthy food products, this doctoral research investigated the alternative structuring approaches for edible liquid oil. Conventionally, liquid oil in lipid-based products is structured using a hardstock that contains saturated and *trans*- fatty acids. These components, however, are associated with the prevalence of non-communicable diseases. Additionally, oleogels have different potential applications such as as delivery vehicles and controlled release. In the latter, the responsiveness of the structure of oleogels provides a tuneable properties that response towards specific external environment factors. Thus, it makes the field of oleogels getting more interesting. Therefore, an alternative structuring technique/approach, *i.e* oleogelation becomes the cornerstone to drive the effort in reformulating lipid based products. Thus, the need for more edible structurants is obvious.

In this study, the focus was given to find synergistic combinations of structurants and techniques in oil structuring. This is due to the fact that some structurants can interact with other structurants, promoting gelation and tuning the oil structuring properties. Additionally, some potential structurants are characteristically insolube in liquid oil, which requires appropriate techniques of incorporation.

The approach employed to find edible structurants (combination) began with extensive random screening of potential molecules and development of preparation techniques (Figure I). Generally, the potential molecules should be edible and have been used as a food ingredient. Meanwhile, the development of a technique involved modification of existing techniques in oleogelation, especially from the indirect approach category. These techniques comprises of melt-mixing, hot and cold-homogenization, and emulsification with the ultimate aim to disperse the potential structurants in sunflower oil (Screening stage).

Once the oleogel is formed, the oleogels were evaluated visually in term of stability (no phase separation) and solidification. On the potential oleogels, a multi-methodological approach was employed to answer the following research questions (Characterization stage):

1. What are the physical properties of the combined structurants?, do the bi-component oleogels exhibit more promising rheological properties than the mono-component systems?
2. What is the resultant effect of interactions?
3. How does the interaction between the structurants inducea change in the oil structuring properties?
4. What are the mesoscopic differences between the oleogels?

The experimental approach was divided into two different parts:

1. Characterization of the behavior of structurants and their combinations
2. Characterization of oil structuring properties of mono-component and bi-component systems

The characterization of the structurants and their combinations offered the initial indicator of possible interaction which is beneficial to elucidate the mechanism of oil structuring. To have a better overview of the potential oleogels, the experimental approach was continued with elucidation of mesoscopic properties. This analysis serves to provide general overview of oleogelation field especially on the possible differences on the molecular level.

Ultimately, this study introduces new structurants and technique to structure sunflower oil. Additionally, the fundamental understanding on the structural formation and synergistic interaction are postulated to get fundamental understanding prior to application in products.

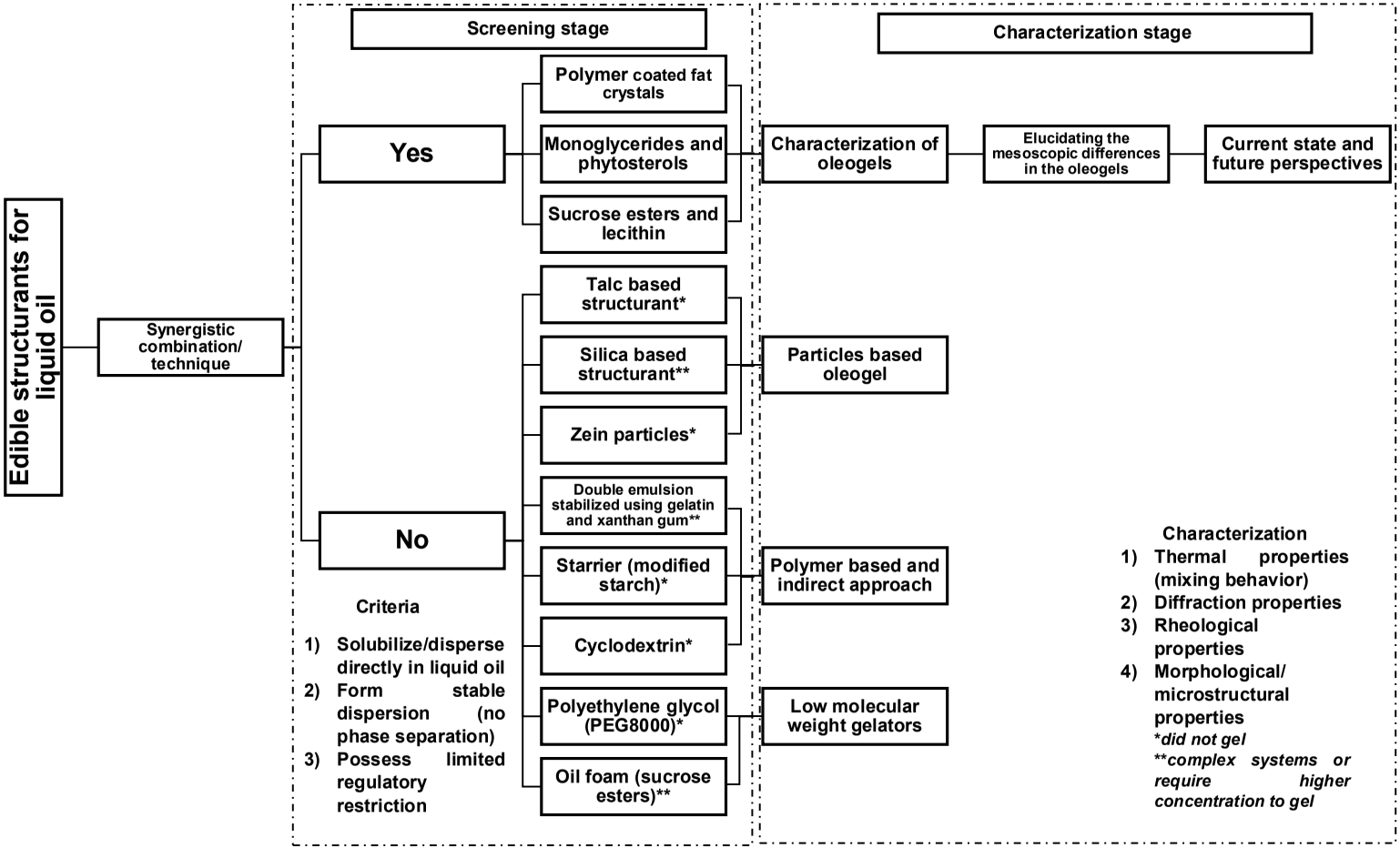

Figure I The approach to the finding of new oleogels

Chapter 1 Oleogelation as an alternative to conventional oil structuring

1 Oleogelation as an alternative to conventional oil structuring

1.1 Background

Gels are soft solids that are widely used in everyday life (Draper & Adams, 2017). Soft gels can be considered as simple models for foods due to the similar underlying network structure, composed of structurant molecules (Vilgis, 2015). Most food gels have water as the continuous medium due to the abundancy of hydrophilic structurants (Vilgis, 2015). Only a limited number of structurants can structure lipophilic media. Still, there is a significant need to structure lipophilic solvents in the food, pharmaceutical, cosmetic and chemical industry. In the last decade, interest in alternative oil structuring has therefore significantly increased, both in academia and in industry (Co & Marangoni, 2012). However, not all oil gelators can be applied in food industry due to additional restrictions, such as safe for consumption, flavor, and specific functional properties (Singh, Auzanneau, & Rogers, 2017).

The need for alternative oil structuring was initially driven to substitute saturated triacylglycerides (TAGs) and *trans* fatty acid (tFAs) in food products. For centuries, TAGs have been used to structure solid-like fat systems (Marangoni & Pink, 2015). Whether TAG-structured systems are solid or liquid at a temperature depends on the chemical nature and physical properties of the constituent fatty acids. Generally, TAGs consisting of mostly saturated fatty acids (SFAs) result in solid fats at room temperature because they crystallize while TAGs containing mostly unsaturated fatty acids do not crystallize at room temperature and result in liquid oil. Liquid oil can be subjected to a hydrogenation process which transforms the liquid oil to semi-solid material. From industrial standpoint, these semi-solid properties are attractive because of the long shelf-life, the stability during deep-frying, and the texturizing effect, which can be customized to enhance the palatability of baked goods, margarine, and sweets (Mozaffarian, Katan, Ascherio, Stampfer, & Willett, 2006; O'Sullivan, Acevedo, Peyronel, & Marangoni, 2015; Wang, Gravelle, Blake, & Marangoni, 2016). However, solid fat prepared via partial hydrogenation contains tFAs, which are associated with the occurrence of non-communicable diseases (NCDs) (Aro, Jauhiainen, Partanen, Salminen, & Mutanen, 1997; Lemaitre, King, Mozaffarian, Sootodehnia, & Siscovick, 2006; Micha et al., 2014; Mozaffarian et al., 2006; Mutanen & Aro, 1997). These NCDs

include coronary heart disease (CHD), type 2 diabetes, obesity, and cancers (Mathers & Loncar, 2006; Micha et al., 2014).

Alternative oil structuring or oleogelation is not just relevant for SFA and tFA substitution. Various other applications could benefit from this innovative field as follows and the detailed are given in the following section of Chapter 1:

1. Product formulation
    a. Solidification or gelation of products such as margarine.
2. Encapsulating materials
    a. Delivery of nutraceutical materials such as β-carotene (O'Sullivan, Davidovich-Pinhas, Wright, Barbut, & Marangoni, 2017).
    b. Modulating physicochemical changes of nutrients; for instance, against oxidation.
3. Controlled release
    a. Responsive oleogels; modulating digestion of TAGs oil (Tan, Peh, Marangoni, & Henry, 2017).
4. Gastronomy;
    a. Oil foam and delivery of oil-soluble flavor components (Rogers et al., 2014).

The main aim of this research was to explore more potential structurants and innovative technique capable of structuring liquid oil (sunflower oil) through extensive screening and development. This is in line with the ongoing expansion and innovation in the oleogelation field (O'Sullivan, Barbut, & Marangoni, 2016; Singh et al., 2017). The main question arises whether the currently available structurants are sufficient to cater the innovation necessary in foods. Each oleogelator appears to have a narrow application field and can be optimized for certain products but not for others. Furthermore, introducing an alternative structurant in a complex food matrix may cause the structurant to lose its functionality during product application, due to interaction with other ingredients (O'Sullivan et al., 2016). More specifically, some structurants are amphiphilic which results in an effect on their functionality when introduced in a complex food matrix, containing water. Thus, the need for more edible structurants are required to bridge the need for more formulation of innovative product in food and other related industries.

Hughes (2009) proposed in a review article the future application of oleogels as delivery vehicles and controlled release based on the fabrication of responsive oleogels, which react to specific environments. This idea stems from the field of the cosmetic and specifically pharmaceutical industry, in which a specific compound is targeted into a specific location with the help of responsive structurants. In the field of chemistry, responsive soft materials are not new. Specific structurants have been chemically synthesized to form gels which have a specific response to environment such as temperature (Liu et al., 2015). To materialize this innovation especially in food industry, ample different types of structurants are required as a different structurant responds differently towards different stress factors. Moreover, the efficiency of the controlled release and delivery vehicles relies on the properties of the structurants such as morphology and the type of self-assembly. However, contrary to research in the field of chemistry, gels for consumer-based products should be safe for consumption. Food-grade structurants are usually found through serendipity based on screening of ingredients. Therefore, this research involves extensive screening of ingredients and improvement of available techniques to alternatively structure liquid oil.

Recently, research also focusses on using oleogels to modulate digestion of lipids (Guo, Ye, Bellissimo, Singh, & Rousseau, 2017; Tan et al., 2017). It is reported that the presence of structurants in oil can act as protective barrier (structuring effect) which delays the exposure of oil to the lipase enzyme during digestion. Moreover, oleogels can delay physicochemical changes in nutrients such as β-carotene, due to also their structuring effect (O'Sullivan et al., 2016).

The interest on oleogelation and its potential application go beyond the initial proposed application, which is to replace SFA in fat based products. The application ranges from structuring alternative, encapsulating agent, to delivery vehicle. Realistically, replacing the TAGs-based structurant is challenging. In food formulations, hardstock helps to retain products in solid form (e.g. margarine) with desirable physical and organoleptic attributes (Acevedo & Marangoni, 2010, 2015; Wang et al., 2016). Reformulating products with alternative fats should have a minimal impact on the organoleptic properties and consumer acceptance. Reformulation requires great effort from the industry and should not be undertaken blindfolded. Prior to product development, a fundamental approach is required to fully understand the potential of the newly developed oleogels (Wang et al., 2016), to tailor with the properties to the potential

application. However, it is easier to said than done as foods are complex systems that contain multiple dispersed phases (van der Sman, 2012; van der Sman & van der Goot, 2009). Additionally, the complexity of food systems is difficult to characterize and requires extensive experimental approach and good instrumentation. These limitations have been discussed and proposed in the concluded Faraday discussion on food structuring. Nevertheless, this is work in progress in the field of food and there is active discussion on this specific field, food physic (Mezzenga, Schurtenberger, Burbidge, & Michel, 2005; Ubbink, Burbidge, & Mezzenga, 2008; van der Sman, 2012; van der Sman & van der Goot, 2009). In other words, to fully understand the complexity of food and to successfully design a product with better qualities (cf: physical, nutritional, and organoleptic) first a fundamental understanding is necessary. Therefore, this study is formulated to find and fundamentally characterize more edible structurants that can structure edible oil to cater the activity in industry specifically food and also pharmaceutical and cosmetic, in which simple gel systems are used.

In this chapter, conventional structuring (TAG-based) will be introduced and discussed. From that understanding, oil structuring is discussed as a soft matter concept. This concept provides a profound contribution in understanding and designing functional structures in chemical and pharmaceutical industries (Ubbink et al., 2008; van der Sman, 2012; Yaghmur et al., 2006). Subsequently, oleogelation is introduced by connecting the oleogel to soft material. Finally, current approaches in oleogelation and its potential application are briefly discussed together with the introduction of the characterization techniques to complete this chapter.

1.2 Conventional approach to oil structuring

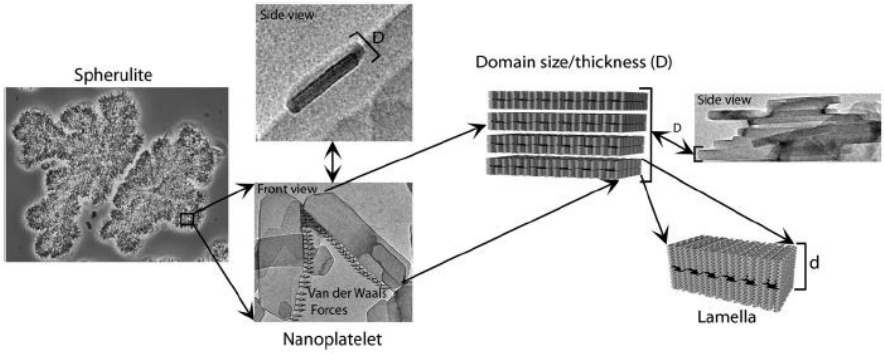

Figure 1.1 Schematic representation of structural levels in a triacylglyceride crystal network, with a platelet characteristic length, width, and thickness (D). At nanoscale, the platelet consists of a lamellar structure with thickness (d). These different length-scales organize to form large spherulite(s), which then closely pack together to form a three-dimensional network (Adapted from (Acevedo & Marangoni, 2010).

Hardstock, such as partially hydrogenated vegetable oil or natural saturated fat, provides a structural building block to structure liquid components in food products. In food products, hardstock contributes to the desirable structural, functional, and sensorial properties of food matrices (Marangoni et al., 2012; Tang & Marangoni, 2006b; Wang et al., 2016). Upon cooling, the saturated and *trans* TAGs have limited solubility in the liquid medium. They self-assemble via nucleation events and grow into small crystals interacting via non-covalent interaction to form a continuous network of crystals (Figure 1.1) (Acevedo & Marangoni, 2010, 2015; Co & Marangoni, 2012; Rogers, 2009; Tang & Marangoni, 2006b).

TAGs crystal networks display a structural hierarchy (Figure 1.2), in which the elasticity is dependent on the interactions between different structural level. In fat-based products (*i.e.*, ice cream, margarine, and chocolate), the TAGs molecules, which exist in a three-dimensional colloidal fat crystal network, determine the physical properties of end products. The physical properties of products are the result of the combined effects of solid content, crystals' size and morphology, thermal behavior, and polymorphic form (Acevedo & Marangoni, 2015; Litwinenko, Rojas, Gerschenson, &

Marangoni, 2002; O'Sullivan et al., 2016; Ramel, Co, Acevedo, & Marangoni, 2016). Since the hardstock TAGs are responsible for the network structure, it is often difficult or impossible to eliminate these ingredients without compromising some of their physical characteristics.

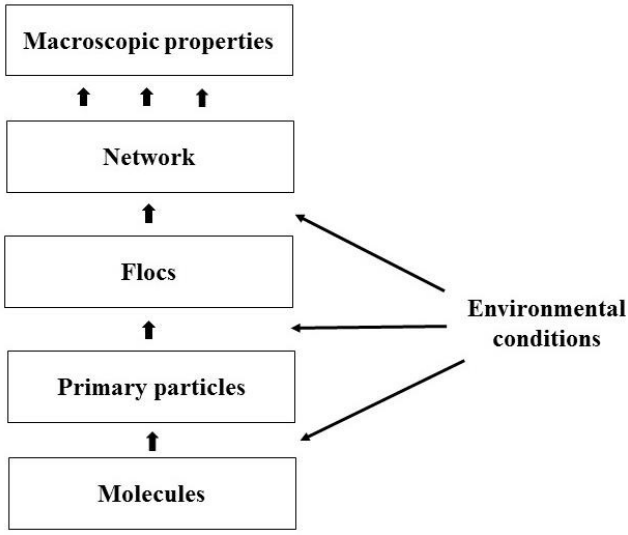

Figure 1.2 Structural hierarchy in many soft food materials that shows the interdependency of different structural hierarhies. This diagram is adapted from (Marangoni & Pink, 2015).

A better understanding of the relationship between the network structure (nano to microscale) of TAGs crystals and the consecutive functionality of the fat, could minimize the use of saturated TAGs and as such result in conforming with the policy on the reduction of SFAs in food products. The TAGs crystallization could be modulated, which would improve the functionality of nanostructure crystals and thus the resultant network (Acevedo & Marangoni, 2015; Marangoni & Rousseau, 1996; Narine & Marangoni, 1999). These nanocrystals and the resultant network appear to have a great influence on the mechanical and rheological properties of lipid-based products (*i.e.*: chocolate, margarine, and shortening). With this approach, reducing the amount of hardstock can be achieved. Despite this positive outcome, consumer perception generally remains negative on the use of saturated TAGs/hard fats, due to association of SFAs with CHD (German & Dillard, 2004; Lawrence, 2013; Wang et al., 2016)

A controlled crystallization process generally results in the formation of nanoplatelet crystals and the subsequent microscale network development. Various researchers have therefore tried to manipulate the crystallization process to improve the functionality of TAGs based structurants. The fatty acid profile of TAGs greatly affects the polymorph and thus also the physical properties (Himawan, Starov, & Stapley, 2006; Vereecken, De Graef, Smith, Wouters, & Dewettinck, 2010). Therefore, TAGs with a specific profile have been developed through chemical interesterification (Marangoni et al., 2012).

Shear and the presence of emulsifiers strongly influence the nucleation and growth of the crystal network formation. These external factors have therefore attracted the attention as means to improve the performance of fat crystal network functionality (Ramel et al., 2016; Sato, Bayes-Garcia, Calvet, Cuevas-Diarte, & Ueno, 2013; Sato & Ueno, 2011; Yoshikawa, Kida, & Sato, 2015). Shear improves heat transfer and nucleation, but can also negatively affect the network formation and functionality if applied excessively (Tran & Rousseau, 2016). Thus, improved functionality of fat crystals is obtained under optimum and controlled shear process (Acevedo, Block, & Marangoni, 2012a, 2012b; Maleky, Acevedo, & Marangoni, 2012). Similarly, addition of emulsifiers enhances and/or delays nucleation and crystal growth of fat crystals (Acevedo et al., 2012b; Smith, Bhaggan, Talbot, & van Malssen, 2011).

Research on fat crystallization has increased understanding on crystal network formation. Yet, the restriction on the use of hardstock in food products implemented in certain countries, forces food scientists and industries to consider structuring methods alternative to conventional TAGs structuring.

1.3 Soft-matter perspectives in edible oil structuring

Designing a structure based on non-TAGs alternatives requires a comprehensive understanding of the colloidal/soft-matter aspects. Colloidal and soft matter principles are essential to understand the behavior of complex materials, and to apply soft matter physics to oil structuring (Mezzenga et al., 2005; Ubbink, 2012; Ubbink et al., 2008; van der Sman, 2012). By definition, soft matter is material that displays solid-like properties but is distinct from traditional materials such as plastics and metals, in that it is more deformable and liquid-like (Gennes, 2005). The microstructure of most soft-matter systems, including foods, is considered to be consisting of hard spheres (Figure

1.3). The hard sphere theory presumes a balance of short-range and long-range attractive and repulsion forces between the molecules (Stokes & Frith, 2008; Ubbink et al., 2008; van der Sman & van der Goot, 2009). In oil structuring, this theory is best translated in the formation of crystal flocs, representing number 5 in Figure 1.3.

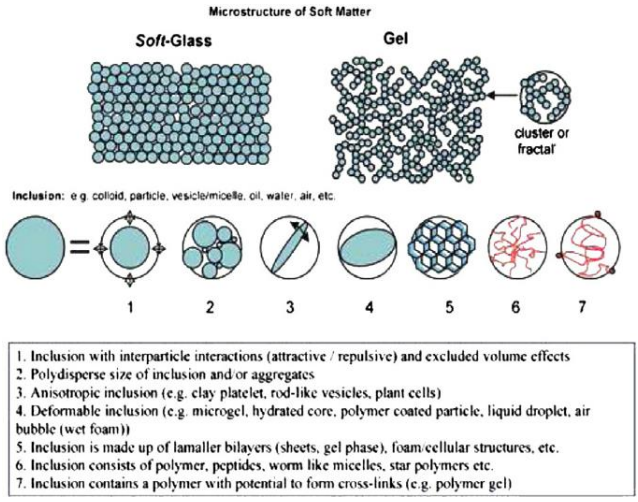

Figure 1.3 Hard sphere concept explaining the microstructure of soft matter (Stokes & Frith, 2008)

Food systems, including lipid-based gels, are considered to be soft-matter, as in many cases the basic building blocks are self-assembled structure with complex phase behavior (Ubbink et al., 2008). All foods are characterized by their length-scale, which has an impact to the nutritional and functional properties (Figure 1.4) (Mezzenga et al., 2005; Ubbink, 2012). The physical properties of the soft matter system largely depend on the product and the relevant length-scales. In emulsion-based system for instance, the relevant scale is the size of emulsified droplets (micrometer scale) (Ubbink et al., 2008).

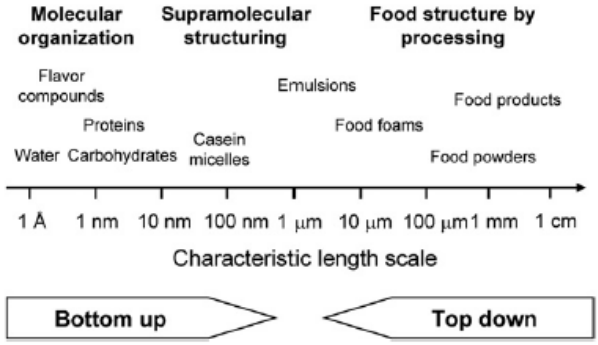

Figure 1.4 Characteristic length-scale of food ingredients and food structures (Ubbink et al., 2008).

Understanding the structure-properties relationships in food can set some general guidelines for rationally designing healthy oil structuring alternatives techniques (Mezzenga, 2007; Mezzenga et al., 2005). The structure-function relationship relies on the thermodynamics and dynamic behavior of complex food materials (Mezzenga, 2007; van der Sman, 2012). This can, for example, be observed in the polymorphic transition of fat crystals. Fat crystals first exist in a metastable state, which is far from equilibrium. Over time, the fat system keeps on exchanging energy with the environment, which consequently induces a transition in the polymorphic form to a stable state.

The organization of food microstructures can be achieved through: (i) self-assembly of specifically designed building blocks (crystallization), (ii) non-equilibrium structure formation by strong external fields (emulsion droplets), (iii) (un)jamming of the food structure (crystal flocs), or (iv) slow dynamics near or in the arrested state (polymorphic transition or high volume fraction of droplets) (van der Sman, 2012; van der Sman & van der Goot, 2009). The presence of solid-like structures such as particle gels or colloidal glasses has been found in conditions where either attractive or repulsion (repulsive or attractive) interactions dominate (Mezzenga et al., 2005; Trappe & Sandkuhler, 2004), but not in an equilibrium state. Any change in the environment renders the system unstable.

Self-assembly is a process in which the components, either as separate (i.e. fatty acids) or linked molecules (i.e. sucrose esters), spontaneously form ordered aggregates (Whitesides & Boncheva, 2002). The requirements to trigger this process are: (1) sufficiently weak (non-covalent) attractive interactions between the structural elements, and (2) the formation of a more ordered state (van der Sman, 2012; Whitesides & Boncheva, 2002). The concept of self-assembly is now attracting attention as a subject of research applying soft-matter theory in food science, in addition to thermodynamic and jamming concepts (van der Sman, 2012).

Different novel structurants have been identified based on their tendency to self-assemble, for instance polar food lipids. Polar lipids are surface active lipids, amphiphilic or low molecular weight emulsifiers (Leser, Sagalowicz, Michel, & Watzke, 2006; Sagalowicz, Leser, Watzke, & Michel, 2006; Sagalowicz, Michel, Blank, Schafer, & Leser, 2017). Self-assembled polar food lipids assemble at mesoscale (~0-100nm) into different structures (Figure 1.5). The resulting structure depends on the type of solvent, concentration, and critical packing parameter of molecules (Leser et al., 2006; Sagalowicz, Leser, et al., 2006; Ubbink et al., 2008). The formed assemblies have multiple functionalities and been applied to encapsulate liquid ingredients, to controll release and reaction media (Leser et al., 2006). The behavior of polar lipids is only one example of obtaining functional structures based on soft-matter concept. This also reflects that amphiphilic compounds in general, have a potential assembly in liquid oil media. In a specific medium, amphiphilic compounds establish strong intermolecular non-covalent interactions that lead to the formation of novel nano-structure assemblies through aggregation (Clemente, Romero, Serrano, Fitremann, & Oriol, 2012; Leser et al., 2006; Wang, Wang, & Zhang, 2012).

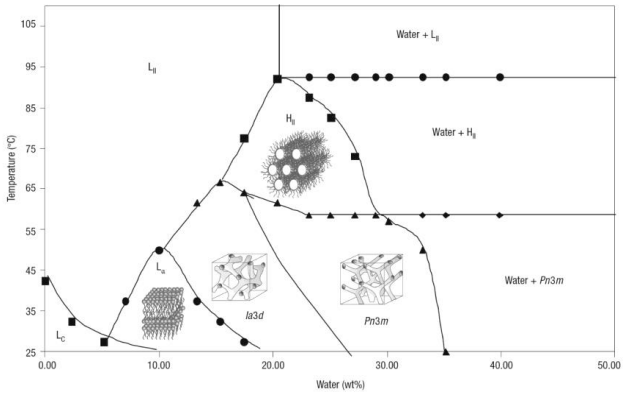

Figure 1.5 Monolinolein in water assembles into different structural architectures, which depends on the concentration and temperature (Mezzenga, 2009).

From a soft-matter perspective, the most interesting approach to reduce tFAs or SFAs in lipid-based products is to physically structure unsaturated vegetable oils (Mezzenga et al., 2005; Ubbink, 2012). Through this approach, the additives provide a scaffold to solidify the liquid oil. Pernetti and co-workers outlined alternatives to structure edible liquid oil: (i) dispersion of a foreign phase (small inert particles, crystallized solids, or/and separated droplets); or (ii) specific molecular mechanisms, such as self-assembly (observed regularly with low-molecular weight organogelators (LMOGs)) (Pernetti, van Malssen, Floter, & Bot, 2007). Figure 1.6 presents the formation of these structuring elements, which act as building blocks for three-dimensional networks with specific functionalities.

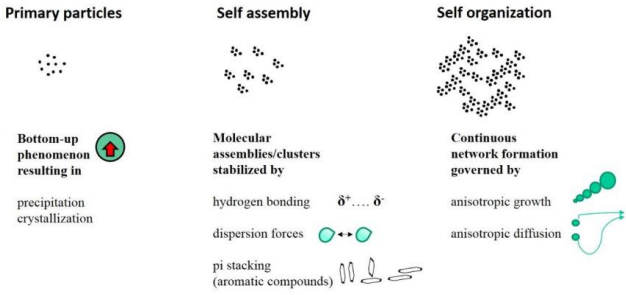

Figure 1.6 The structural hierarchy in the formation of oleogels from nanoscale to micro/macro scale level (Patel, 2017).

Soft-matter research has aided in the development of characterization techniques for soft materials such as lipid-gel. Nano-scale assembly and interactions involved during crystallization of monoglycerides have been studied using X-ray diffraction and Fourier Transform Infrared Spectroscopy (Chen & Terentjev, 2009; Lupi, Greco, et al., 2016; Sagalowicz, Mezzenga, & Leser, 2006). Furthermore, the relationship between fractal dimension of crystals and rheological properties was investigated using Polarized Light Microscopy (PLM), rheology, Ultra-Small Angle X-ray Diffraction (USAXD), and Electron Microscopy (Acevedo & Marangoni, 2010; Peyronel, Pink, & Marangoni, 2014; Peyronel, Quinn, Marangoni, & Pink, 2014; Tang & Marangoni, 2006a, 2006b). Interestingly, a combination of understanding and characterization techniques permits the creation of potential alternative systems for edible oil structuring. In general, soft matter concepts, and more specifically self-assemblies, offer interesting and safe routes to structure edible liquid oil (Sagalowicz et al., 2017).

1.4 Introduction to an oleogel

An oleogel or organogel, depending on the solvent used in the preparation, is a viscoelastic system in which a non-polar solvent (edible liquid oil) is immobilized by a three-dimensional network formed by self-assembled molecules or gelators (Abdallah, Sirchio, & Weiss, 2000; Abdallah & Weiss, 2000; Co & Marangoni, 2012; Hughes, Marangoni, Wright, Rogers, & Rush, 2009; Patel & Dewettinck, 2016; Sahoo et al., 2011). This self-assembled structure is formed by the entanglement of one or more structuring units such as crystals, fibrillar networks (SAFiNs), or suspended polymer strands (Patel & Dewettinck, 2016). The gelators or structurants are mostly organic molecules that spontaneously self-assemble to form a three-dimensional network (Figure 1.6). The network is formed by cooling the warm solution of gelator in organic solvent (edible oil) below the gelation transition temperature (Terech & Weiss, 1997).

Oleogels show similar behavior to soft materials, as they respond towards changes in the external environment such as pressure, temperature, etc. (Weiss, 2014). Formation of an oleogel involves different stages, which begins with self-assembly at nanoscale level up to network formation at microscale level, alike a soft material (Figure 1.6) (Mezzenga et al., 2005; Patel, 2017).

1.5 Current approaches to oleogelation

Oleogelation has attracted the interest from food scientists, engineers, and industries as a potential alternative for conventional TAGs fat structuring. A number of recently published review articles summarize the current state of oleogelation (Bot, Veldhuizen, den Adel, & Roijers, 2009; Co & Marangoni, 2012; Dassanayake, Kodali, & Ueno, 2011; Patel & Dewettinck, 2016; Pernetti, van Malssen, Floter, et al., 2007; Rogers, 2009).

The quest to find other edible structurants is challenging because structurants that typically create good oleogels are limited to regulatory approval. Despite of that, different approaches and techniques have been discovered recently, which spans from mono-component to mixed combination oleogels. The structurants can vary widely, but mainly fall under the categories of crystalline particles, polymeric strands, particle-filled networks and liquid crystalline mesophases (Nikiforidis & Scholten, 2014; Patel & Dewettinck, 2016) (Figure 1.7).

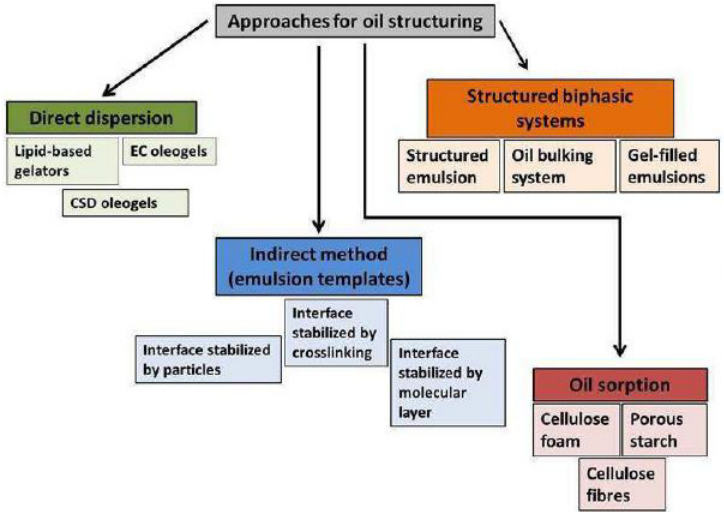

Figure 1.7 Current state of oleogelation is divided into several different categories based on the structural building blocks. This chart is obtained from Patel et.al., 2016.

In this section, the described approaches are screened from a wide variety of oleogelation techniques, to have comprehensive view on current state of edible

oleogels, which are limited to food applications. In general, oleogelation is divided into categories based on the type of preparation, source of structurant, and molecular arrangement (Figure 1.7).

The type of structurants and oleogels have profound effects on their potential applications. Specifically for the delivery of drugs and functional ingredients, the type of structurants has a big impact. In other words, specific type of oleogel is best suited for specific application (McClements, 2008; McClements, Decker, Park, & Weiss, 2008; O'Sullivan et al., 2016). However, for food applications, there is no general guidelines available for the selection of oleogels and usually through random selection. This is understandable because oleogelation is considerably new for food application. Therefore, most of researchers dealing with reduction of fat in food products only consider the structuring ability of oleogels. This is partly due to the lack of fundamental understanding on oleogels. In each category, structuring mechanism is explained briefly to gain understanding of the system.

1.5.1 Crystalline-based oleogelation

Crystalline-based oleogelation is widely studied in edible structuring due to its resemblance to the conventional structuring. Conventional structuring with a crystalline TAGs phase (hardstock) becomes the benchmark for a crystalline-based oleogel (Bot, Veldhuizen, et al., 2009) (Figure 1.8). In contrast to the conventional structuring, the crystalline particles are formed from non-TAG molecules. Generally, the molecules of a structurant self-assemble into lamella, which then stack together to create space-filling network, during cooling from melt to a temperature below the crystallization temperature (Bot, Veldhuizen, et al., 2009; Dassanayake et al., 2011). Due to the resemblance with TAGs-based structurant, considerable number of crystalline-based structurants have been discovered thus far, as summarized in the following section. Moreover, as the structure relies on the crystallization of molecules, the post crystallization events have an influence on the properties of resultant oleogels. In monoglyceride oleogels, for instance, crystal aggregation caused by the formation of β-polymorph compromises the gel properties. The approaches to control the crystallization of fat could be extrapolated to crystalline based oleogels to mitigate the problems associated with post crystallization. There are numerous studies performed to control the crystallization process of crystalline based oleogels to improve the oil

structuring properties, such as shear, cooling rate, and combination system (ethylcellulose) (Lopez-Martinez, Charo-Alonso, Marangoni, & Toro-Vazquez, 2015).

Figure 1.8 Microstructure of crystals network of palm stearin (a), monoglycerides (b), and combination of rice bran wax and sunflower wax (c) structured edible liquid oil using polarized light microscope.

1.5.1.1 Monoglycerides

The use of monoglycerides as structuring agents has gained significant interest due to their ability to gel both aqueous and non-aqueous solvents (Sagalowicz, Leser, et al., 2006). The crystallization of the fatty acid alkyl chain can be achieved either through supersaturation or supercooling of monoglycerides in oil dispersions (Co & Marangoni, 2012; Meng et al., 2014; Ojijo, Neeman, Eger, & Shimoni, 2004). Notably, saturated monoglyceride has shown the promising ability to form elastic gels in liquid oil through the formation of crystalline network (Bin Sintang, Rimaux, Van de Walle, Dewettinck, & Patel, 2017; Chen & Terentjev, 2009; Da Pieve, Calligaris, Panozzo, Arrighetti, & Nicoli, 2011; Lopez-Martinez et al., 2014).

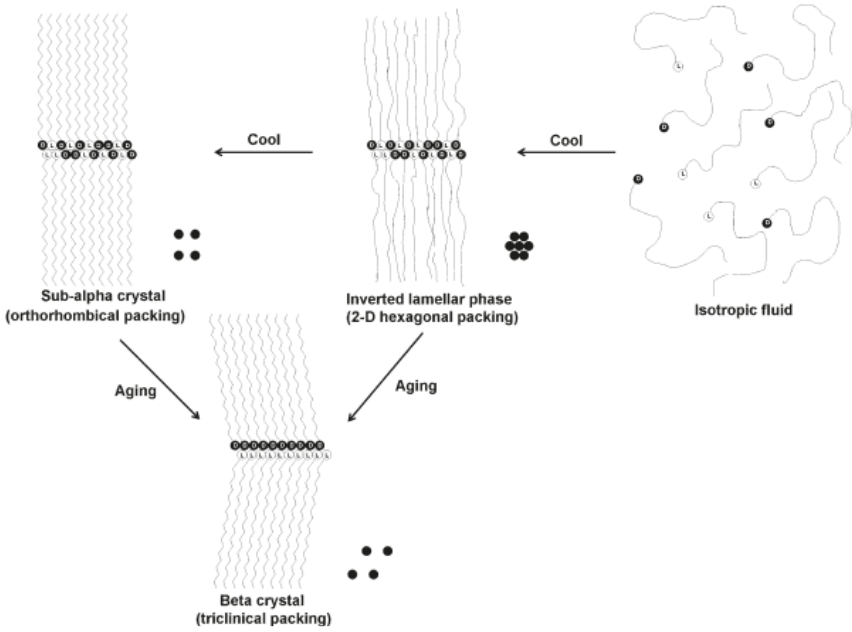

Figure 1.9 Structural formation of monoglyceride crystals in a hydrophobic solvent (Chen & Terentjev, 2009).

Gelation and crystallization of monoglycerides are outlined through the formation of four generic phases: isotropic, inverse lamellar, sub-α crystalline and β-crystalline phases (Chen & Terentjev, 2009), as shown in Figure 1.9. Since both the crystallization and mesoscopic organization of monoglycerides are significantly affected by the processing conditions, the physical characteristics of monoglycerides-based gels are largely dependent on parameters such as cooling rate, concentration and shear that can be controlled during preparation (Cerqueira et al., 2017; Da Pieve, Calligaris, Co, Nicoli, & Marangoni, 2010; Ojijo et al., 2004).

1.5.1.2 Waxes

Waxes are mixtures of straight-chain alkanes, long-chain fatty acids (FAs), long-chain fatty alcohols (FALs), and wax esters (WEs) (Asperger, Engewald, & Fabian, 1999). Long-chain alkanes in specific and other constituents present in waxes form microplatelet crystals during crystallization. The crystals then aggregate to form a “card-house” microstructure, forming a three-dimensional network (Figure 1.10). The

molecules are packed into the orthorhombic polymorph with some traces of the monoclinic polymorph (Abdallah et al., 2000).

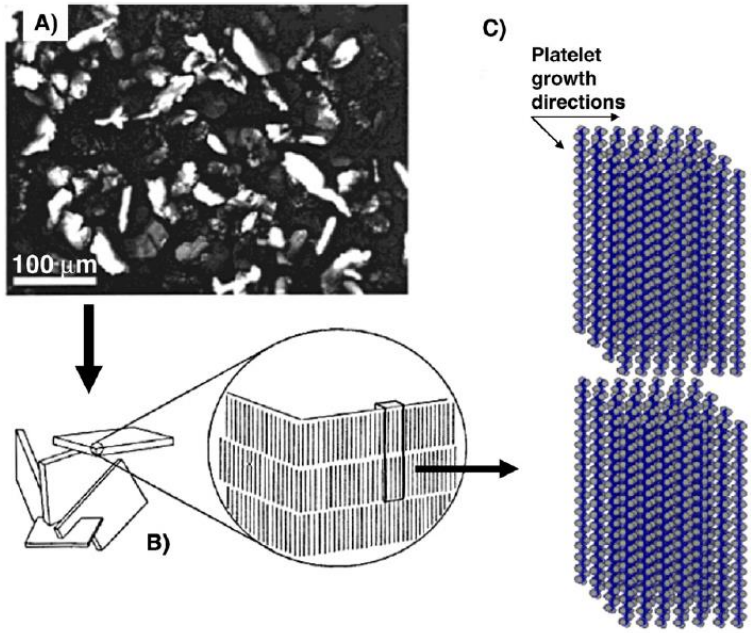

Figure 1.10 Straight-chain alkanes assemble into microplatelet crystals, which then aggregate to form an expansive “card-house” microstructure (Abdallah et al., 2000).

Wax can be obtained from different sources, resulting in a wide variety of chemical compositions. Different research groups have extensively investigated oleogelation of different waxes in different types of oils. Oleogels have been formed using candelilla wax, rice bran wax, fruit wax, beeswax (Doan, et al., 2015; Tavernier, et al., 2017), shellac (Patel, et al., 2013), carnauba wax (Doan et al., 2015; Toro-Vazquez et al., 2007), sunflower wax (Doan et al., 2015; Jana & Martini, 2016; Martini, et al., 2015; Tavernier, et al., 2017), and paraffin wax (Jana & Martini, 2016; Martini et al., 2015).

1.5.1.3 Fatty acids and fatty alcohols

Fatty acids and fatty alcohols show a similar structuring mechanism as n-alkanes, forming multilamellar structures (Figure 1.10). However, the latter generally has a longer chain than the fatty acids and fatty alcohols used in oil structuring (Co & Marangoni, 2012). Gelation using fatty alcohols and fatty acids therefore requires

higher concentrations of structurant than waxes (n-alkanes). Daniel and Rajasekharan showed that the minimum gelling concentration of fatty acids changes with the change in chain length (Daniel & Rajasekharan, 2003).

Synergistic interactions are observed in mixtures of fatty acids and fatty alcohols. In edible oil, the mixture at 3:7 (stearic acid:stearyl alcohol) formed a harder gel than its corresponding mono-component. The mono-components also exhibit different morphologies than their combination. The combination displays needle-like crystals while the mono-components show plate-like shapes. The formation of needle-like crystals is attributed to a mixed crystal system, which has higher order reflection in SAXD, indicative of thin crystals (Blach et al., 2016; Gandolfo, Bot, & Floter, 2004; Schaink, van Malssen, Morgado-Alves, Kalnin, & van der Linden, 2007). The formation of a mixed crystal system with needle-like morphology is responsible for the improvement of hardness of the combination oleogel.

Recently, Nikiforidis and co-workers gelled a liquid oil using the combination of oleic acid and sodium oleate. Oleic acid when dispersed in edible oil shows no structural formation due to its high solubility in oil. Addition of sodium oleate (at ratio 1:1) causes disturbance in the intermolecular hydrogen bonding, leading to formation of fine crystals having an inverse lamellar arrangement (Nikiforidis, Gilbert, & Scholten, 2015). This arrangement is responsible for the gelation of oleic acid-sodium oleate mixture in liquid oil.

1.5.2 Self-assembled fibrillar networks/low molecular weight oleogelators

Low molecular weight oleogelators (LMOGs) has shown to assemble into a structure identical to a polymer network. The LMOGs self-assemble into a fibrillar network of specific dimensions due to their geometrical packing. Their entanglements create a space-spanning three-dimensional network, entrapping solvents in the nanospaces (Buerkle & Rowan, 2012; Dastidar, 2008; Nikiforidis & Scholten, 2014; Raghavan & Douglas, 2012; Terech & Weiss, 1997). Small organic compounds with a molecular mass typically lower than 3000 dalton are superior in immobilizing organic solvents and pure water at very low concentration of gelator (as low as 1 %) (Dastidar, 2008; Terech & Weiss, 1997; Vintiloiu & Leroux, 2008; Weiss, 2014).

To date, only a few edible LMOGs have been identified. These include oryzanol-phytosterol (Bot & Agterof, 2006), long chain saturated fatty acid-saturated fatty alcohol (Schaink et al., 2007), ricinelaidic acid (Wright & Marangoni, 2007), lecithin-sorbitan tristearate (Pernetti, van Malssen, Kalnin, & Floter, 2007), and lecithin-tocopherol (Nikiforidis & Scholten, 2014) for edible application. Controlled crystallization of Phytosterols produce a fiber-like structure (SAFiNs) and grow unidimensional. This is in contrast to TAGs and monoglycerides in which the crystalline particles growth multidimensional (Figure 1.11). Thus, finding a self-assembled structurant that is edible and can form into self-assembly fibrillary networks (SAFiNs) provides opportunities which allow extensive trial-and-error application study.

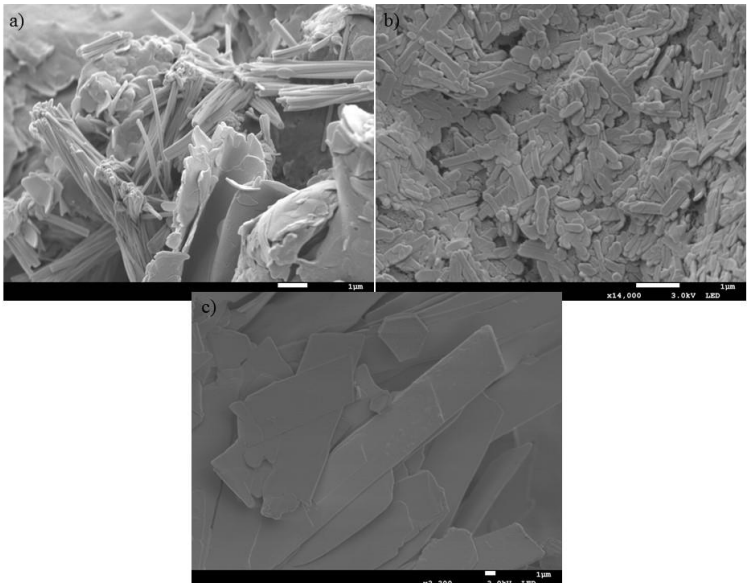

Figure 1.11 Relative comparison of a difference between fiber-like structure of phytosterols in the presence of monoglycerides (a), plate-like structure of TAGs (b), and plate-like structure of monoglycerides (c).

Basically, this polymeric-like structure elongates in one dimension and overlaps, forming a transient network. The network provides a mesh structure that can immobilize edible liquid oil. Some researchers propose that assembled networks of rod-like or tubule structure from a small quantity of gelator is found to be most efficient to immobilize large volumes of liquid oil (Co & Marangoni, 2012; Terech & Weiss, 1997;

Vintiloiu & Leroux, 2008). Basicly, it is difficult to found such gelators that could assemble into SAFiNs structure and edible. This is contrary to the field of chemistry where gelators with specific chemical structure that assembled into SAFiNs could be simply synthesized (Weiss, 2014).

1.5.2.1 Surfactant-based structurants

In organic non-polar solvents, surfactants can assemble into different architectural structures (Clemente et al., 2012; John, Zhu, Li, & Dordick, 2006; Molinier et al., 2006; Shchipunov & Shumilina, 1995; Stubenrauch, 2001), which are formed through hydrogen bonding or other non-covalent interactions (Buerkle & Rowan, 2012; Clemente et al., 2012; Dastidar, 2008; Terech & Weiss, 1997). This ability arises from the amphiphilic nature of surfactants. Additionally, amphiphilic surfactants forming assemblies are prone to react to any changes in the environment (Leser et al., 2006; Stubenrauch, 2001; Wang et al., 2012).

A considerable number of surfactant-based gelators have been found to be able to structure edible oil. Lecithin in combination with certain other molecules are capable to self-assemble into tubular-like structures. Among the combinations are lecithin with tocopherols (Nikiforidis & Scholten, 2014), lecithin with water (Bodennec, Guo, & Rousseau, 2016), and lecithin with sorbitan tri-stearate (Pernetti, van Malssen, Kalnin, et al., 2007), which all gelled edible liquid oil at various concentrations and ratios. The gelation is the result of a change in the self-assembly which modifies the structural organization of lecithin from spherical micelles to tubular micelles. This transition was caused by a disruption in the intermolecular hydrogen bonding of lecithin in the presence of an additive molecule, affecting the curvature angle (Figure 1.12) (Bodennec et al., 2016; Nikiforidis & Scholten, 2014; Scartazzini & Luisi, 1988; Shchipunov, 2001; Shchipunov & Shumilina, 1995; Shchipunov, Shumilina, Ulbricht, & Hoffmann, 1999; Vintiloiu & Leroux, 2008).

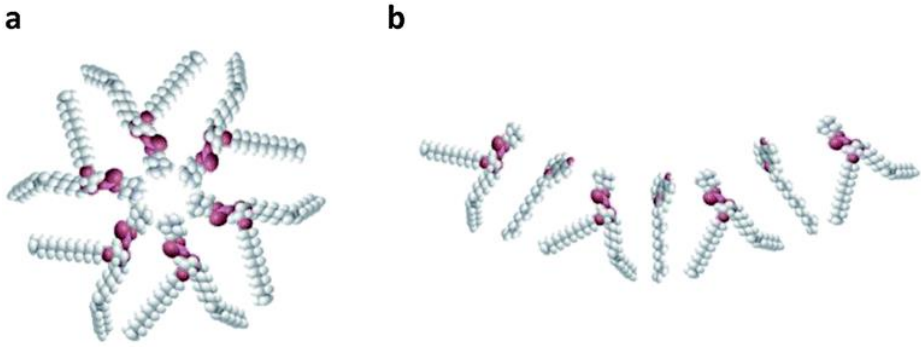

Figure 1.12 The curvature angle of self-assembled lecithin (a) and in the presence of tocopherol (b) in edible oil (Nikiforidis & Scholten, 2014).

1.5.2.2 12-Hydroxystearic acid

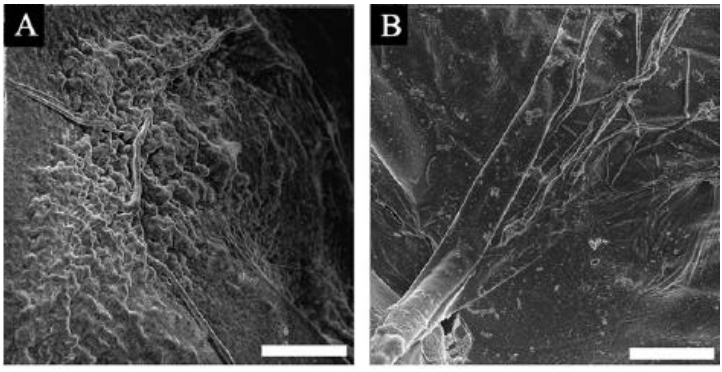

Figure 1.13 12-hydroxystearic acid crystallizes and elongates into one-dimensional crystal structure. This structure overlaps and forms a transient network, responsible for gelation of edible liquid oil, scale bar = 100μm (Rogers, Wright, & Marangoni, 2008).

Hydroxylated fatty acids and their soap derivatives have been shown to have excellent oil-structuring capability, for example 12-hydroxystearic acid (Lan & Rogers, 2015; Rogers et al., 2008). 12-Hydroxystearic acid (12-HSA) forms tubules/fibrillar structure in edible oil, as depicted in Figure 1.13 (Kuwahara, Nagase, Endo, Ueda, & Nakagaki, 1996). This structure forms due to the lack of conformational freedom, which forces the hydroxyl group on 12-HSA to form a zigzag intermolecular hydrogen bonds network. The structure elongates into certain length and starts to branch. The structural

organization depends on gelator-gelator, gelator-oil, cooling rate, and water content (Lan & Rogers, 2015; Rogers et al., 2008).

1.5.2.3 β-Sitosterol and γ-Oryzanol

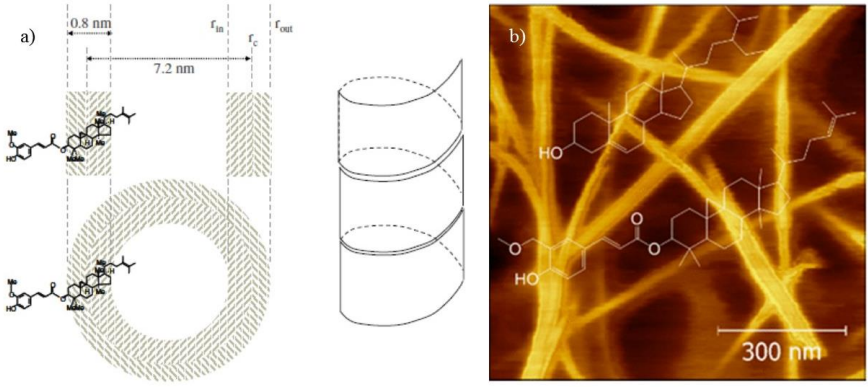

Figure 1.14 The structure of tubules from the combination of β-sitosterol and γ-oryzanol combination, illustrated from SAXD analysis (Bot, den Adel, Roijers, & Regkos, 2009) (a) and visualized under Atomic Force Microscope (Matheson, Koutsos, Dalkas, Euston, & Clegg, 2017) (b).

Phytosterols and phytosterol esters are present in trace quantities inside the cellular membrane of plant cells and vegetable oils (Vaikousi, Lazaridou, Biliaderis, & Zawistowski, 2007). Mixtures of β-sitosterol or any other un-esterified sterols with γ-oryzanol have shown to create firm oleogels (Bot & Agterof, 2006; Bot, den Adel, & Roijers, 2008; Bot, den Adel, et al., 2009). The oleogels were translucent in appearance, indicating that the microstructural components are smaller than the wavelength of visible light, which is associated with the formation of nano-scale tubules in vegetable oils. The dimension of these tubules was 6.7 to 8.0 nm in diameter and the wall thickness varied between 0.8 to 1.2 nm, as can be seen in Figure 1.14 (Bot et al., 2009; Matheson et al., 2017).

The tubules are formed from the interaction between the hydroxyl groups at carbon-3 of β-sitosterol and carbonyl group of γ-oryzanol. This interaction leads to the stacking of sterol ring in a helical manner, forming the wall of tubules (Figure 1.14) (Bot et al., 2008; Bot, den Adel, et al., 2009; Matheson et al., 2017). It has also been reported that hydroxyl group of phytosterols is important for gel formation (Bot & Agterof, 2006).

However, the structuring ability is compromised in the presence of water due to the interference in the hydrogen bonding by water molecule (Sawalha et al., 2012).

1.5.3 Polymer-based structurant

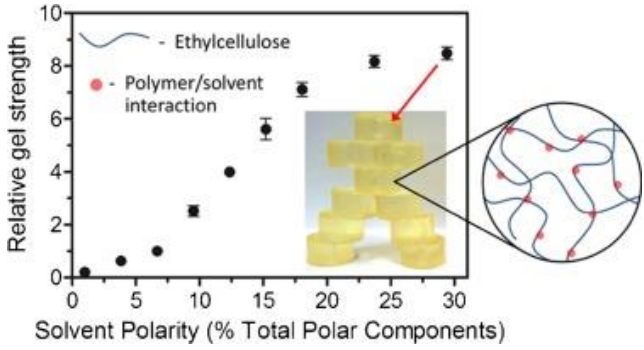

Figure 1.15 Formation of ethylcellulose crosslinks below the set temperature is responsible for gelation of edible oil (Gravelle, Davidovich-Pinhas, Zetzl, Barbut, & Marangoni, 2016).

Ethylcellulose (EC) is derived from cellulose in which the hydroxyl groups have been ethylated and the degree of substitution (DoS) determines its solubility, (i.e., 1.0 < DoS < 1.5: soluble in water; 2.4 < DoS < 2.5: soluble in organic solvents) (Davidovich-Pinhas, Barbut, & Marangoni, 2015). Ethylcellulose (EC) is the only known polymer that can be dispersed directly in edible liquid oil. However, EC requires heating to temperatures above its glass transition temperature and shearing at this temperature for uniformity of dispersion (Davidovich-Pinhas, Barbut, et al., 2015; Davidovich-Pinhas, Barbut, & Marangoni, 2016). The dispersion turns to gel upon cooling below the set point, which depends on the solvent polarity (Figure 1.15) (Gravelle et al., 2016). At this temperature, the polymeric chains associate via hydrogen bonding (Davidovich-Pinhas, Gravelle, Barbut, & Marangoni, 2015). Despite of the required high temperature, which causes fat oxidation, the group of Marangoni has extensively studied EC oleogels. Their studies span from fundamental of gelation to applications in real food systems (Barbut, J. Wood, & A. Marangoni, 2016a, 2016b; Barbut, J. Wood, & A. G. Marangoni, 2016c; O'Sullivan et al., 2016). This oleogel also belongs to cellulose fibers because it is cellulose derivative.

1.5.4 Particle-based structurant

Colloidal silicium dioxide (CSD) particles have been shown to gel sunflower oil. High shear (<10000 rpm) was needed to transform the mixture of sunflower oil and CSD to gel at room temperature. This process allowed de-agglomeration of CSD aggregates, which uniformly dispersed and formed a continuous network upon removal of shear. The oleogel showed substantially higher elastic modulus than loss modulus (Patel, Mankoc, Bin Sintang, Lesaffer, & Dewettinck, 2015). However, 15 wt% of CSD in oil was required to form firm gel.

1.5.5 Indirect approach

Comparing the function of a hydrocolloid in a hydrogel and an oleogelator in an oleogel, both serve a similar role: providing a matrix to contain a liquid phase. However, there are more structurants for hydrogels than for oleogels because more structurants solubilize in water than in oil (Patel & Dewettinck, 2016). Polymers containing a hydrophilic and hydrophobic part have surface active properties, and can stabilize emulsions (Nasatto et al., 2015). Therefore, the surface activity of polymers is a potential route to incorporate them into hydrophobic solvents via an indirect approach, as shown in Figure 1.16 (Dickinson, 2009; Hayakawa, Kawaguchi, & Kato, 1997).

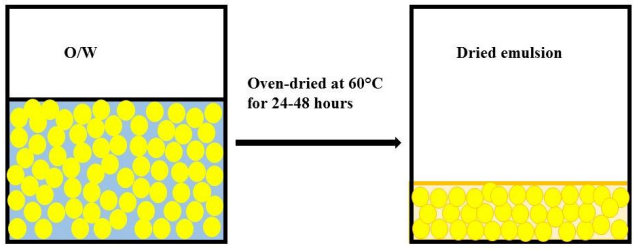

Figure 1.16 The oil-in-water emulsion (a) is oven dried to remove water from the continuous phase. The resultant dried emulsion (b) is now consist only polymer network and oil, which is sheared to obtained gel-like properties (Romoscanu & Mezzenga, 2006).

1.5.5.1 High internal phase emulsion

Using concentrated emulsion droplets, a biphasic system consisting of aqueous and oil phases can be immobilized. This type of system is widely applied in the preparation of mayonnaise and other sauces, where closely crowded dispersed droplets at high

packing fraction (volume fraction > 0.74) immobilize the biphasic system (Patel & Dewettinck, 2016).

High internal phase emulsions (HIPE) gel sunflower oil as the continuous phase. In a study by Patel and co-workers, galactomannans, carrageenan, and xanthan gum were used to gel the aqueous dispersed phase. Due to high phase volume, the gelled droplets provided a structural framework that held the biphasic system together (Patel, Rodriguez, Lesaffer, & Dewettinck, 2014). This type of oleogel involve the addition of gelled aqueous phase (droplets) that filled the system and hence sometimes called as gelled filled emulsion (Figure 1.7). Additionally, the system composes of two biphasis system of oil and water simultaneously. This system sometimes is considered belong to indirect approach as well. In this study, the system is described under the indirect approach.

1.5.5.2 Emulsion-templated approach

The emulsion-templated approach has gained considerable attention as a route to structure edible oil using hydrophilic polymers. The hydrophilic polymer provides the structural network, as it adsorbs at the interface during the preparation of oil-in-water emulsions. Then, the emulsion is dried to evaporate the aqueous phase, leaving the polymer network at the interface encasing oil. The group of Mezzenga was the first to apply this approach for edible oil structuring (Romoscanu & Mezzenga, 2006). Recently, the group of Dewettinck has used different polymers in either single or combination forms, which resulted in numerous oleogels from different polymers network (Patel, Cludts, Bin Sintang, Lesaffer, & Dewettinck, 2014; Patel, Rajarethinem, et al., 2015; Romoscanu & Mezzenga, 2006; Tavernier, Patel, Van der Meeren, & Dewettinck, 2017).

Moreover, de Vries and co-workers applied stepwise solvent exchange method to prepare oleogels using the same basic approach as in the emulsion-templated approach (de Vries, Hendriks, van der Linden, & Scholten, 2015; de Vries, Lopez Gomez, Jansen, van der Linden, & Scholten, 2017; de Vries, Wesseling, van der Linden, & Scholten, 2017). The aqueous phase is removed through solvent-exchange instead of drying. In their study, a protein hydrogel was first prepared and the encased solvent (water) was exchanged with liquid oil, using acetone or tetrahydrofuran as

intermediate solvent (de Vries et al., 2015; de Vries, Wesseling, et al., 2017). However, the use of organic solvents limits its application in foods.

The emulsion-templated approach therefore offers a higher potential as a route to exploit structuring ability of hydrophilic polymers in edible oil, since solvents are not required.

1.5.5.3 Foam-templated approach

The foam-templated approach is used to fabricate an oleogel based on hydrophilic polymers. Patel and co-workers exploited the surface activity of hydroxyl propyl methylcellulose (HPMC) by fabricating an aqueous foam, which was subsequently freeze-dried to create a porous cryo-gel. Due to oil sorption property of this gel, liquid oil could penetrate the porous and as such an oleogel was prepared (Patel, Schatteman, Lesaffer, & Dewettinck, 2013). Recently, κ-carragenaan was used to prepare a cryo-gel as the template for edible oil structuring (Manzocco et al., 2017). The compounds that are capable to stabilize air bubbles are scarce. Additionally, the formed template should have oil sorption properties. Due to these limitations, this approach is not really popular. However, its potential applications are big, since aerogel (cryogel) is also used to control crystallization of molecules (Ubeyitogullari & Ciftci, 2016).

1.5.6 Oleofoam

Lipid-based foams, described as oleofoams, are rarely encountered as opposed to aqueous foams. Lipid foams can be stabilized by molecular or particulate emulsifiers (Binks & Marinopoulos, 2017; Fameau & Saint-Jalmes, 2017; Gunes et al., 2017). Oleofoams have recently gained considerable attention as a mean to structure liquid oil, transforming an oleogel to oleofoam. The transformation of oleogels to oleofoam is achieved through whipping the solid oleogel at constant speed and time. Some researchers described this system as a whipped oleogel (Fameau et al., 2015; Gunes et al., 2017).

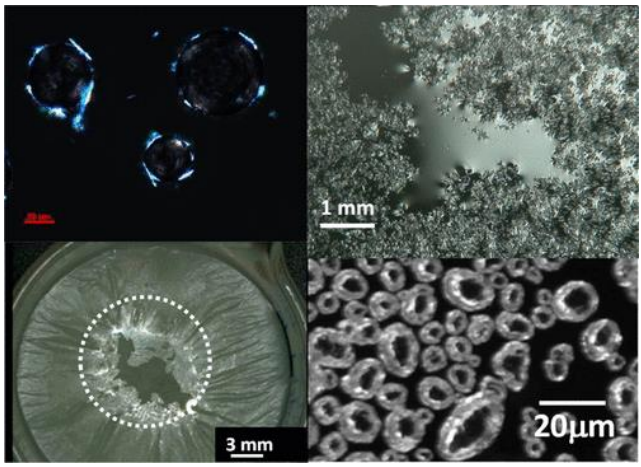

Figure 1.17 A jammed interfacial crystal layer of monoglycerides stabilized air bubbles, leading to the formation of oleofoam (Gunes et al., 2017).

The air bubbles in a whipped oleogel are stabilized by crystals, creating a Pickering effect at the oil-air interface (Figure 1.17). The crystals from the oleogel form a jammed, closely packed layer of crystals around the bubbles (Binks & Marinopoulos, 2017; Fameau et al., 2015; Gunes et al., 2017). Oleofoams have been prepared using monoglycerides (Gunes et al., 2017), fatty alcohols (Fameau et al., 2015), and TAGs (Binks & Marinopoulos, 2017; Mishima, Suzuki, Sato, & Ueno, 2016).

1.6 Applications of oleogels

The ability of oleogels to structure liquid oil make them an interesting alternative for conventional TAG structuring. Although the basis of oleogelation is still being thoroughly researched, considerable research on its application have already been conducted. Oleogels appear to have wide application potential in food, pharmaceutical and chemical industries. An oleogel can be either applied directly or in combination with other gelation systems, creating hybrid or bigel systems. The use of oleogels in food products depends on the type of structurant, preparation, and aim. In the following section, a selection of oleogel applications as a structurant, delivery agent, and controlled release agent are discussed.

1.6.1 As a structurant and co-structurant

Oleogels are attractive as a potential alternative to conventional TAG-based oil structuring, reducing SFAs and tFAs in lipid-based products (Wang et al., 2016). This potential stems from their oil binding capacity and ability to immobilize oil which turn liquid oil to solid-like systems. In consumer-based products such as cosmetic and pharmaceutical, instead of acting as structuring agent, oleogels are used to deliver functional ingredients or drugs which will described later. In food-related application tests, there is no systematic approach for selection of oleogels for specific product application. This is because it is generally believed that oleogels are also capable of providing a structuring effect similar to SFA in edible oil.

1.6.1.1 Chocolate and confectionaries

Alternative structurants have been applied in chocolate and confectionary products both to reduce oil migration in products and to reduce the level of saturated fats.

The ability of an oleogel to reduce oil migration, inducing fat bloom in baked cakes product was patented by Nakano and Masaki (Nakano & Masaki, 1989). They used disaturated-monounsaturated mixed triacylglycerides combined with a minimum of one long chain saturated fatty acid residue as the structurant at 10 wt% in oil. The mixture showed gel-like properties which resisted mobility and migration of oil. Similarly, monoglyceride and sorbitan-tristearate oleogels were used as a co-structurant in the filling of pralines, which resulted in a delay in oil migration (Si, Cheong, Huang, Wang, & Zhang, 2016).

Doan and co-workers (2016) combined beeswax in rice bran oil with palm oil to reduce the level of saturation in a hazelnut filling for praline chocolate. This combination resulted in a complex crystallization behavior, producing different crystal morphologies capable of imitating the physical properties of the reference filling (Doan et al., 2016). Patel and co-workers also combined shellac oleogel in chocolate paste formulation to reduce the saturation level (Patel, Rajarethinem, et al., 2014).

Rice bran wax oleogel was used in ice-cream to substitute milk fat. A high degree of fat structuring was observed within the fat droplet, associated with the crystal morphology of rice bran wax. Formation of a better structure with rice bran wax in

combination with glycerol monooleate has shown a potential as a co-structurant in ice-cream (Botega, Marangoni, Smith, & Goff, 2013).

1.6.1.2 Margarines and shortenings

Margarines and shortenings are products containing high levels of SFAs. A considerable number of studies have therefore been performed to investigate the application of oleogels as a (co-)structurant in margarines and shortenings. The main challenge is to obtain products with similar properties as products with a conventional formulation. Replacing a shortening with wax and emulsion-based oleogels in respectively cookies and cakes resulted in improvement in spreadability of cookies' dough with soft physical properties and retention of similar volume expansion (porosity) of cakes compared to the reference (Jang, Bae, Hwang, Lee, & Lee, 2015; Patel, Cludts, Bin Sintang, Lesaffer, et al., 2014; Patel, Rajarethinem, et al., 2014). However, a high concentration of wax oleogels in dough was required to compensate the loss of structuring properties from shortening (Kim, Lim, Lee, Hwang, & Lee, 2017; Mert & Demirkesen, 2016a, 2016b). Still, substituting shortening with wax oleogel in the formulation helps to improve nutritional properties of the end-product whilst substantially maintain the sensory properties. The baked products containing oleogels exhibit almost identical sensory properties (Patel, Rajarethinem, et al., 2014; Yilmaz & Ogutcu, 2015a, 2015b). Patel and co-workers conducted sensory study and found that cake containing shellac oleogels-shortening had identical sensory properties with the reference (shortening only). However, differences were found on the cell size and crumbliness, due to uneven distribution of air in batter containing shellac oleogel (less solid fat that can stabilize the air) (Patel, Rajarethinem, et al., 2014).

1.6.1.3 Meat and related products

A considerable amount of research has been performed on the application of oleogels as fat replacer in meat products. The formulation of Italian sauces was modified by incorporating structurants such as monoglycerides and lecithin to its conventional formulation of using only liquid oil. The researchers found that addition of the oleogel imparted a stabilizing action (Lupi et al., 2012), due to structuring effect by the structurants. Ethylcellulose and sorbitan monostearate are also used as a fat replacer in sausage. These structurants modified the juiciness, but no significant difference in hardness was found relative to reference (Barbut et al., 2016a, 2016b; Barbut et al.,

2016c). By using a combination of monoglycerides' and phytosterols' oleogel, Kouzounis and co-workers partially replaced pork backfat in frankfurter formulation. The researchers found a decrease in hardness in frankfurter containing oleogel compared to reference sample, yet with similar elasticity (sensory) as the reference (Kouzounis, Lazaridou, & Katsanidis, 2017).

1.6.1.4 Non-food products

Oleogels are applied in several fields such as the cosmetic industry and the crude oil industry. In this section, only the applications of oleogels in cosmetics are discussed because it is a consumer-based product. Cosmetic products use oleogels as a strategy to prevent sweating, a condition in which a film of oil forms on the surface of the product (Co & Marangoni, 2012). This phenomenon originates from the migration of liquid oil to the surface by capillary or diffusion action, which is also responsible for fat bloom in chocolate products.

1.6.2 Delivery vehicle/agent

For pharmaceutical-based products, oleogels can be applied as delivery agents because most pharmaceutical products require a solid matrix for the delivery of active drugs and to indirectly structure a liquid phase. Oleogels can be used as agents for both topical and oral delivery. The main advantage of topical delivery is pertaining the degradation effect of drugs by the action of gastric juices and enzymes, which is commonly observed in oral administration. Therefore, topical delivery possesses advantages over oral delivery, but requires a matrix that can overcome the barrier properties of the stratum corneum. Some oleogels have shown to be good permeation enhancers, such as lecithin-based oleogels (Sahoo et al., 2011; Vintiloiu & Leroux, 2008). Lecithin-based oleogel presented several favorable characteristics for transdermal delivery owing to their amphiphilic nature (Raut et al., 2012; Vintiloiu & Leroux, 2008). The delivery of aceclofenac from lecithin-based oleogel was found to have better release pattern and consistency through the skin layer (Shaikh, Jadhav, Gide, Kadam, & Pisal, 2006).

Due to unique structural organization of SAFiN-based oleogels, which form colloidal or fibrillar networks, attempts have been made to investigate the feasibility of these oleogels as oral delivery agent and as controlled release agent of functional materials

in foods (O'Sullivan et al., 2016; Vintiloiu & Leroux, 2008). Still the effective delivery of lipid soluble compounds through oleogelation can be challenging. These challenges arise from the matrix effect of oleogel, the solubility of functional materials in the matrix, and chemical stability of functional materials (Norton, Espinosa, Watson, Spyropoulos, & Norton, 2015; O'Sullivan et al., 2016; Sandra, Decker, & McClements, 2008).

In cosmetic products, Kirilov and co-workers gelled sunscreen (Vaseline) oil using the emulsification approach. In their study, colloidal nanoparticles of sunscreen (Vaseline) oil were produced, forming gelled particles. As a result, a sunscreen cream with improved photoprotective ability and photostability of UVB filter was developed (Kirilov et al., 2014). The oleogel was capable of simultaneously providing structure to the cosmetic product and of encapsulating bioactive material in the oil phase (Glampedaki & Dutschk, 2014).

There is less research reported on the application of oleogels as a delivery vehicle in foods. Recently, β-carotene was dissolved in different solid matrices (tristearin, tripalmitin, and saturated monoglycerides) and the results showed that oleogelation was able to deliver and protect β-carotene and improve its stability against oxidation (Calligaris, Valoppi, Barba, Anese, & Nicoli, 2014; O'Sullivan et al., 2017). In another study, the oxidative stability of cod-liver oil was doubled when dispersed in a monoglyceride oleogel (Da Pieve et al., 2011).

1.6.3 Controlled release of functional ingredients and drugs

There is clear distinction between controlled release and delivery applications of oleogels. Both applications rely on the matrix of oleogel to contain ingredients or drugs internally. However, the matrix used for controlled release is stimuli-responsive to certain environmental changes such as pH and enzyme activity (Sangeetha & Maitra, 2005; Vintiloiu & Leroux, 2008). This behavior permits the delivery and release of ingredients or drugs at specific locations. Controlled release agents are mainly useful in the pharmaceutical and cosmetic industry, where a substance must be targeted to a specific location (Guo, Wang, Cao, Lee, & Zhai, 2010; Hughes et al., 2009; Vintiloiu & Leroux, 2008).

A lecithin-pluronic acid oleogel was investigated for its controlled release and structuring ability of aloe vera (*Aloe vera linné*) and Hydrocotyle asiatica (*Centella*

asiatica) in cosmetic products for the treatment of cellulite (Morales, Gallardo, Clares, Garcia, & Ruiz, 2009). Lupi and co-workers showed the degree of structurant in policosanol/olive oil oleogel affected the release of ferulic acid in an *in vivo* study (Lupi et al., 2013), intended for cosmetic application. In pharmaceutical, 12-hydroxystearic acid oleogels containing ibuprofen was used for controlled release of drugs. The researchers found that erosion and dissolution rate of oleogel and theophylline were the main factors governing the drug release (Iwanaga, Kawai, Miyazaki, & Kakemi, 2012).

In formulations involving a lyotropic liquid crystalline phase of monoglycerides and oleoyl glycerides, the latter showed higher resistance towards the digestive process. Consequently, cinnarizine-incorporated oleoyl glycerides structures had a bioavailability three times higher due to monoglyceride mesophases. The drug was released and adsorbed periodically over 120 hours shown in their animal study (Boyd, Khoo, Whittaker, Davey, & Porter, 2007).

In food, the digestive process of oleogels is interesting in research towards scale-up of oleogels to real food products. Hughes and co-workers conducted a clinical trial to investigate the post-prandial increase in TAGs, free fatty acids, glucose and insulin after ingestion of structured and unstructured product lipid products. The researchers found that the increase in free fatty acid and TAG levels following ingestion of canola oil oleogel (as a spread) was lower compared to the group that consumed butter or margarine (Hughes et al., 2009). A similar effect was found on the glucose level and TAG profile in a study conducted with ethylcellulose oleogel (prepared in coconut oil) (Tan et al., 2017). In a different study by Duffy et al., oleogels were found to affect the digestion of lipids, decreasing the TAG lipolysis. The study was performed *in vitro* by simulating the duodenal digestion of oil-water emulsion in which the dispersed phase was structured with phytosterols (Duffy et al., 2009). These studies prove the ability of the oleogel to control the digestion process in term of enzyme activity and thereby improve the lipid profile. Although this application was not yet fully studied in food systems, there is potential to engineer and develop a responsive oleogel (Hughes et al., 2009; O'Sullivan et al., 2016).

1.7 Characterizing the properties of oleogels

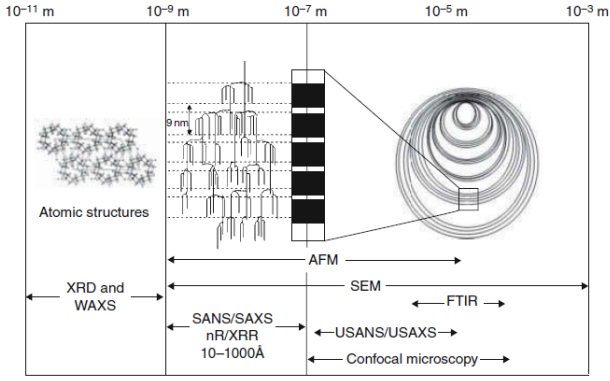

Figure 1.18 Depending on the relevant length-scale, different characterization techniques can be applied (Lopez-Rubio & Gilbert, 2009).

In general, the physicochemical, sensory, and nutritional properties of oleogels are determined by the type of components present, their interactions, and their structural organization. In oleogels, the structure at macroscale is formed by a lower scale building block, resulting in structural hierarchy (Figure 1.2 and 1.4). This structural hierarchy is determined by different forces at different length-scales (nano-scale, micro-scale, or macro-scale). Figure 1.18 depicts the correlation of length-scale to the type of characterization techniques. The structure and dynamics of complex food systems have traditionally been investigated by techniques widely employed in experimental soft condensed matter physics.

Concepts from material science, soft-matter and food engineering are the basis for the selection of the characterization techniques. The selected techniques provide an understanding on the performance of oleogels, covering their physical properties and building block architecture (self-assembly) (Figure 1.18). The oleogels are characterized as follows:

1. Rheological properties
2. Thermodynamic properties
3. Self-assembly/lattice properties
4. Microstructural properties

1.7.1 Rheological properties

Oleogels can be considered as soft matter. Therefore, it is decisive to elucidate their physical performance. Due to the complex response of any soft material to deformation, the physical performance is best described with rheology (Stokes & Frith, 2008). Rheology is the study of the deformation and flow behavior of matter. Rheological properties vary between viscous fluids and elastic solids (Fischer, Pollard, Erni, Marti, & Padar, 2009; Fischer & Windhab, 2011; Mezger, 2011). A soft material such as an oleogel has properties varying between ideal liquids and ideal solids. It exhibits both elastic and viscous responses and is therefore called viscoelastic. This leads to complicated deformations in response to mechanical stress (Chen, Wen, Janmey, Crocker, & Yodh, 2010; Stokes et al., 2008; Stokes & Frith, 2008).

Small amplitude oscillatory (SAO) tests have been systematically used to characterize viscoelastic behavior of gels (Chen et al., 2010; Rao, 2007). In these tests, a sinusoidal oscillatory stress or strain with a defined frequency (Hz) is applied to the material to measure the phase difference between oscillatory stress and strain. In dynamic tests, a gel is subjected to a small sinusoidal oscillating strain or deformation. The applied strain generates two response components in viscoelastic materials: an elastic component in line with the strain and a 90° out of phase viscous component. For deformation tests within the linear viscoelastic region (LVR), the results are expressed in terms of elastic or storage modulus (G') and viscous or loss modulus (G") (Mezger, 2011; Rao, 2007).

Scaling relationship is a theory used to explain the elastic properties of colloidal gels by considering the structure of the gel network as a collection of close packed fractal flocs of colloidal particles (Shih, Shih, Kim, Liu, & Aksay, 1990). The theory describes two separate rheological regimes: (i) strong-link regime is observed in low particle concentrations, and (ii) weak-link regime is observed at high particle concentrations. The weak-link regime is applicable to gels that are well above the gelation threshold. The scaling relationship model has been used to link the rheological properties of fat-based products to their fractal dimension. Fractal dimensions have shown to more significantly contribute to rheological properties than the solid fat content.

1.7.2 Thermodynamic properties

Calorimetry is a technique which measures the thermal properties of materials as a function of temperature. The technique establishes a relationship between some specific physical properties of molecules by measuring the transition temperature and the associated enthalpy of transition. Hagemann and Rothfus used differential scanning calorimetry (DSC) to study TAGs' polymorphism (Hagemann & Rothfus, 1983). DSC has since systematically been employed to study the phase transition in fats. Additionally, DSC has been used as a preliminary identification of polymorphs of fat crystals (Foubert, Fredrick, Vereecken, Sichien, & Dewettinck, 2008), where X-ray diffraction is not available. Hence, DSC shows to have great potential in studying the phase transition in oleogels (Doan et al., 2017; Lan & Rogers, 2015; Rogers et al., 2008).

1.7.3 Self-assembly/Organization properties

In elucidating the molecular assembly in an oleogel, diffraction analysis is the most useful tool. In principle, when a beam of X-ray is directed to a material, some of the radiation will be absorbed, some will be diffracted in a new direction with or without a change in energy and the remainder will pass through the material unaffected. Diffraction of X-rays from atoms arises through interaction with the electrons and generates secondary spherical waves that emanate from the electron. Since crystals have regular atomic positions, a regular array of waves is generated. The constructive interference results in reflections or diffraction peaks. Hence, the molecular arrangement/assembly can then be determined using Bragg's law (Gilbert, Lopez-Rubio, & Gidley, 2015).

Powder X-ray diffraction (powder XRD) is one of the best methods to study the solid state of crystals and lipid mesophases (Deman, 1992; Fanun, Wachtel, Antalek, Aserin, & Garti, 2001; Marangoni et al., 2012). The usual information reported are the long spacings (Small Angle X-ray diffraction; SAXD) and short spacings (Wide Angle X-ray diffraction; WAXD) of crystals which define their polymorphic form. X-ray diffraction patterns can also provide additional and valuable information on lipid mesophases (Acevedo & Marangoni, 2010; Marangoni et al., 2012; Nikiforidis, 2015). However, the power of the radiation source limits the application of powder X-ray diffraction to identify the assembly of structuring oleogel molecules at low structurant

concentrations. Fortunately, the advancement in radiation science helps to identify such low concentration system through the introduction of synchrotron radiation.

Synchrotron radiation is polychromatic, with a peak in the X-ray region for an electron energy 7.5 GeV. The radiation is generated from the acceleration of electron (Rosenbau.G et al., 1971). Synchrotron radiation possesses advantages that a normal X-ray source could not have. Among the advantages are a high signal/noise ratio and a high angular resolution which allow the analysis of complex structural details and the observation of complicated peak broadening effects (Cheng et al., 2017). In food-related application, synchrotron radiation has been widely employed to investigate the arrangement of emulsifier at the emulsion's droplet interface (Wassell et al., 2012), the effect of emulsifier on the bulk fat (Verstringe, Dewettinck, Ueno, & Sato, 2014), and effect of tempering on the partial coalescence (Moens, De Clercq, Verstringe, & Dewettinck, 2015) to name a few.

1.7.4 Microstructural properties

The microstructural properties of oleogels influence their physical performance. Microscopic techniques provide the opportunity to visualize the basic structural architecture, which also become the tools that contribute to the emerging research in food structure (Smith, 2015). Visualization of food structure provides thorough knowledge of the structural organization of the main components and their relation to bulk properties, such as texture, stability and appearance (Gilbert et al., 2015).

Several visualization techniques have been identified and applied to characterize the nano/microstructure of oleogels. Optical microscopy equipped with a polarization filter is widely used to visualize the microstructure of fat crystal network, which is modelled with the fractal dimension (Tang & Marangoni, 2006b). Fractal dimension of crystal network has been correlated with the rheological properties of lipid-gel (Marangoni & Rousseau, 1996; Tang & Marangoni, 2006b). Polarized light microscopy is an important microscopic technique in the study of fat crystallization to probe for different crystal morphologies at submicroscopic level. This is based on the birefringence property of fat crystals. In fat crystals, the molecular order induces optical anisotropy which cause the absorption, refraction, and scattering of light to be dependent on the orientation of the material (Mehta, Shribak, & Oldenbourg, 2013). Recently, advances

in the imaging technique allows visualization at nanometer scale using both scanning and transmission electron microscopy (Acevedo & Marangoni, 2010; Smith, 2015).

In a biphasic system, the use of confocal laser scanning microscopy has shown to provide valuable information. Confocal laser scanning microscopy is an imaging technique used to increase micrograph contrast and/or to reconstruct three-dimensional images by using a spatial pinhole to eliminate out-of-focus light in specimens which are thicker than the focal plane (Gilbert et al., 2015). Confocal microscopy has been widely used to characterize the microstructure of emulsions, where the aqueous and oil phases are contrasted using specific dyes.

1.8 Perspectives

Most structurants are found through serendipity and for several applications, no good solution or approach has been found yet (Co & Marangoni, 2012; Singh et al., 2017; Terech & Weiss, 1997; Weiss, 2014). In this research, new structurants have been researched for their application potential, particularly in food industy but also in pharmaceutical, cosmetic, and chemical industries. Therefore, the aim of this research was to develop new oleogels based on different structurants using extensive screening and exploration. During the screening stage of potential structurants, 10 to 12 wt% of total concentration was used to prepare oleogels, which considerably lower than other studies. From the extensive screening of food ingredients and exploration of new oleogelation techniques, the following chapters describe the new structurants and techniques to structure edible liquid oil (Figure 1.19). In essence, this research explored the combination of structurants in search of synergistic interactions, as specific interaction between molecules are able to tune the self-assembly and hence gel properties. The synergistic interaction of combined structurants do not only offer interesting properties that lead to gelation but also provide fundamental insight in the interaction. Therefore, among the formed oleogels at the screening stage (Figure I), the oleogels formed through the combination of structurants were selected. Additionally, the complexity of double emulsion and oleofoam, and regulatory restriction of colloidal silica particles are the basis to exclude those systems from detailed investigations and as such from this dissertation.

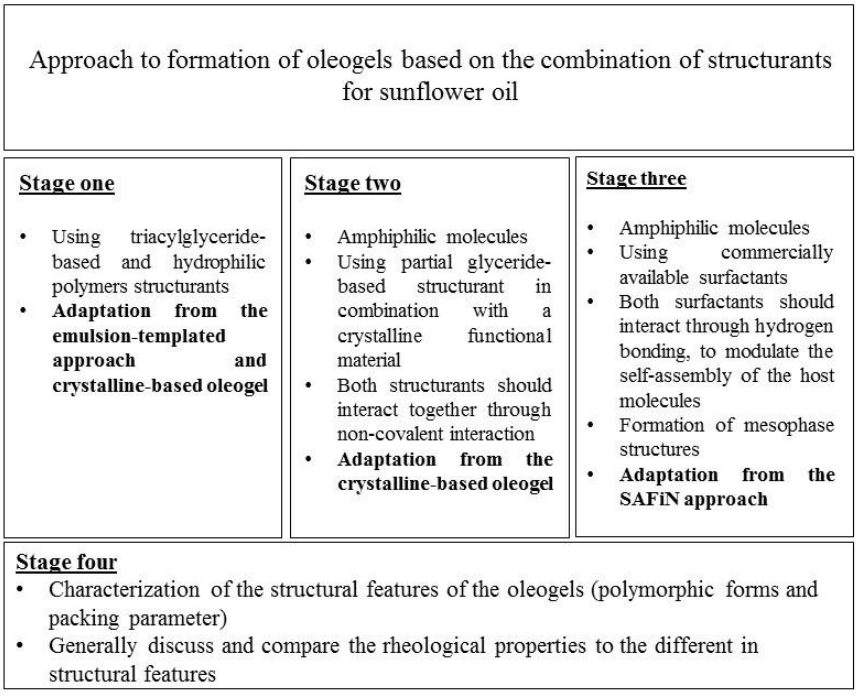

Figure 1.19 The summary of the approaches to discover the synergistic combination of structurants.

Edible liquid oil is non-polar, while most gelators are polar. Therefore, an innovative technique is needed to incorporate hydrocolloids into oil medium, giving the fact that most of hydrocolloids need to be hydrated to form a network. This can be achieved through indirect approach of incorporation of hydrocolloids into liquid oil. Therefore, Chapter 2 describes and introduces an innovative approach of delivery of hydrocolloids using prepared fat capsules thus, that Chapter serves as a proof-of-concept of developed approach. Hydrocolloids such as methylcellulose and gelatin are commonly used structuring agents that can form good gel in water, and they are surface active. Due to their surface activity, these hydrocolloids can adsorb at the interface of an oil in water emulsion. In emulsions, these hydrocolloids form a stabilizing network or protective layer coating the dispersed oil phase, and act as a carrier by crystallizing the dispersed phase, forming fat capsules in powder form. This can be achieved with crystallized fat such as fully hydrogenated rapeseed oil (FHRO) as the dispersed phase, but potentially also with other crystallizing materials. FHRO consists of high

melting triacylglycerides used commonly as a crystal starter in margarine and a crystal stabilizer to prevent oiling out in chocolate paste (Peyronel, Campos, & Marangoni, 2016). The proposed innovative approach here is not only applicable for fabrication of fat capsules for oil structuring but also for the production of solid lipid nanoparticles with improved functionality (Kim & Vanapalli, 2013) and with broad potential applications (O'Sullivan et al., 2016; Scalia, Young, & Traini, 2015). The melted fat capsules as a structuring agent led to the formation of a composite oleogel, which relies on the structuring effect mainly from fat crystals and an additional effect from polymer strands. Scientifically, the presence of colloidal aggregates such as polymer network in this study induces jamming (volume) effect to oleogel. The proposed new concept of jamming is widely studied in soft matter and just recently tested in fat crystals network. This concept offers modification of the physical properties of fat-based system and hence limits dependency towards the use of emulsifiers or interesterification. Ultimately, Chapter 2 introduces new innovative approach of delivering hydrophilic polymers to sunflower oil through the fabrication of fat capsules. Additionally, the chapter also describes the application of the fat capsules as a structuring agent. Chapter 2 provides a proof-of-concept research on the approach to deliver hydrophilic polymer.

Monoglycerides have been used extensively as structuring alternative to the conventional system. However, studies have shown that monoglycerides, especially saturated monoglycerides, produce large crystal which compromise their structuring ability (Chen & Terentjev, 2009). Contrary to the approach reported in literature whereby shear and ethylcellulose were used to control the crystallization of monoglycerides (Da Pieve et al., 2010; Lopez-Martinez et al., 2015), this research utilized phytosterols. Chapter 3 describes the application of phytosterols to influence the crystallization and as such the rheological properties. The selection of distilled monoglycerides containing almost 40 wt% of unsaturated fatty acid (oleic acid) provides additional advantage to this research since most of the reported studies utilize saturated monoglycerides. Industrially, phytosterols in esterified form are added into margarine to improve its nutritional value. The use of esterified phytosterol in margarine is due to its low melting temperature compare to the unesterified phytosterols (Acevedo & Franchetti, 2016), which the latter produce a product with gritty texture. Additionally, the high melting temperature of unesterified phytosterols limit their digestion in human

thus, losing their functionality to reduce serum blood cholesterol (Acevedo & Franchetti, 2016; Engel & Schubert, 2005; Salo & Wester, 2005; Vaikousi et al., 2007). It has been reported in literature that emulsifiers such as monoglycerides and lecithin can influence the crystallization of phytosterols and hence reducing the melting temperature of unesterified phytosterols. Therefore, this study not only investigates the oil structuring properties of monoglycerides and phytosterols combinations, it also investigates the effect on the crystallization particularly the effect of phytosterols on monoglycerides. This combination is inspired by nature, in which sterols influence the physical properties of lipid membrane, condensing effect or chain ordering, and translated into the oil structuring concept. Ultimately, Chapter 3 describes and discusses the role of phytosterols in influencing the crystallization of monoglycerides by inducing chain ordering to achieve improvement in the oil structuring properties.

The ability of amphiphilic molecules to assume different assemblies provides interesting alternative to conventional oil structuring. However, the formation of such building blocks requires random screening and combination of amphiphilic surfactants, especially those with low hydrophilic-lipophilic balance (HLB) value. The low HLB surfactants are easier to dissolve in liquid oil and do not require the assistance of additional solvent such as water to ease solubilization as in the case of high HLB surfactants (Hashizaki, Taguchi, & Saito, 2009; Szuts, Pallagi, Regdon, Aigner, & Szabo-Revesz, 2007). This research successfully developed oleogels based on the combination of sucrose esters (HLB-2) and sunflower lecithin. The formation of the oleogels was mainly due to the self-assembly modification in sucrose ester by sunflower lecithin. The discovery of oleogels based on sucrose esters and sunflower lecithin combinations expands the number of edible structurants capable of forming SAFiNs like structure, which are currently limited. Most of the current structurants capable of forming SAFiNs require higher concentration (Bodennec et al., 2016). The oleogels produced in this research were based on a total concentration of only 10 wt% and without the addition of ethanol. Contrary to microemulsion and the formation of wormlike (tubular) micelles, ethanol or other polar solvent is required to form stable system (Garti, Clement, Leser, Aserin, & Fanun, 1999; Hashizaki et al., 2009). Instead, the sucrose esters and the combinations with lecithin were melted separately prior to the addition of hot sunflower oil thus, preventing the aggregation of the structurants. This approach eliminates the need of polar solvent as bridging agent that functioned

to prevent aggregation (Nikiforidis & Scholten, 2014). Chapter 4 discusses and describes the oleogels formed, based on the lipid mesophase structures of amphiphilic molecules.

A thorough understanding of the nanostructure assembly of an oleogel is important to characterize and define the final structure. Therefore, the nanostructure assembly of the reported oleogels were characterized by using synchrotron X-ray diffraction in Chapter 5. This powerful instrument has been widely used to characterize the nanostructure assemblies of soft-matters and biological structures (*i.e*: protein). The study of nanostructure assemblies of the oleogels provides fundamental information to upscale the oleogels to real product. For instance, the conventional oil structuring relies on the lamellar stacking, while pharmaceutical and cosmetic industries mainly employ the mesophase structures (*i.e*: worm-like micelles and cubic structure).In addition, Chapter 5 serves to unravel the synergistic effects of the studied oleogels especially on the molecular organization. The information on the effect at the molecular level is important as the assembly or crystallization at the nanometer length-scale will influence the macroscale properties. Additionally, with the selection of structurants consisting of TAGs-based and in the presence of polymer, monoglycerides and phytosterols combinations, and sucrose esters and lecithin combinations permits a direct comparison of complex modulus between the different group of structurants having different molecular organizations. In other words, direct comparison between the complex modulus, molecular organization, and type of structurants in oleogels can be achieved.

In general, this research serves to fundamentally unravel the synergistic interactions between the combined structurants and the concept governing the developed technique. The information and knowledge provided by this research allow a more targeted approach to upscale oleogels to the industrial stage. Innovation is paramount in food industry and the key to produce high quality food products. Considering broad and innovative applications of organogels or oleogels (depending on the type of solvent) in cosmetic, pharmaceutical, and chemical industries, crossed disciplinary approach and concept could be applied to upscale oleogels in food industry. The potential applications of oleogelation in food industry are still at the stage of extensive development and are mostly investigated as an alternative for SFAs and tFA. Exploring

the potential applications of oleogels in real food system requires fundamental understanding on their behaviors and properties at simple system (oil medium).

Oleogels are formed mostly based on the spontaneous self-assembly of molecules forming a three-dimensional network. A soft matter, particularly a food gel, is one of the most complex soft matter types with multiple dispersed phases and hierarchical structure. This provides an explanation why there is no systematic or even scientific approach that could predict oleogelation behavior of a specific molecule, especially in the case of amphiphilic molecules. Although most oleogels contain only oil as the disperse phase (direct approach), yet, they comprise of hierarchical structure (Figure 1.2). Therefore, this study only focused on oleogelation properties of screened structurants in sunflower oil to mitigate the complexity of gel and allow a better understanding on the gel formation. The fundamental understanding from this study provides the industries information to justify and fit the oleogels into their specific products.

Chapter 2 Polymer coated fat crystals as an oil structuring agent

Relevant publication:

Bin Sintang, M.D., Tavernier, I., Danthine, S., Van de Walle, D., Doan, C.D., Muhammad, D.R.A., Rimaux, T., & Dewettinck, K. Polymer coated fat crystals as an oil structuring agent. **Will be submitted to Food Hydrocolloids.**

2 Polymer coated fat crystals as an oil structuring agent

2.1 Research strategy/hypothesis

The aim of this chapter was to develop an alternative approach to incorporate hydrophilic polymers to sunflower oil. Therefore this chapter serve as a proof-of-concept that describes and discusses the innovative method of polymer coated fat crystals (fat capsules) as the carrier of hydrophilic polymers. The developed approach involve a series of preparation steps of emulsification, creaming, and drying. In this chapter, crystallized oil of fully hydrogenated rapeseed oil (FHRO) was used in an oil-in-water emulsion as the dispersed phase (oil droplets) which crystallized inside the droplets and produced fat capsules coated with hydrophilic polymer. Hydrophilic polymers are known to be good structurants in water, but could not be functioned as structurants due to difficulty to dissolve them in edible oil. However, some hydrophilic polymers are surface-active, and hence can adsorb at the water-oil interface. The selection of FHRO as the crystallized oil in this chapter is due to its excellent crystallization properties that crystallizes quickly thus, facilitating the development of polymer coated fat crystals. However, other crystallized species might be explored too or even in the mixture with oil. Fast crystallization of FHRO permits the formation of discrete spherical fat capsules coated with polymer. Additionally, in the production of margarines, shortenings and other fat-rich food poducts, FHRO is commonly used as a crystal starter to influence the fat crystallization.

The formed fat capsules were then added to sunflower oil, heated to melt the fat capsules and hence release the adsorbed polymer to sunflower oil. Upon cooling, the FHRO (from the fat capsules) crystallizes to form fat crystals network, whilst the polymer sheets (film or network) suspend randomly and trapped between the fat crystals. It is hypothesized that the presence of polymer networks between the fat crystals network induces jamming or volume effect and hence improving the rheological properties of oleogels.

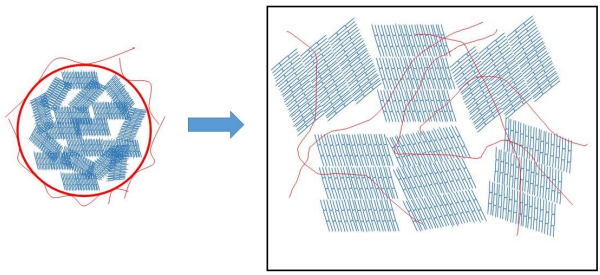

Figure 2.1 Schematic illustration of the polymer coated fat crystal and its behavior in an oleogel system, delivering hydrophilic polymer to edible oil. The internal fat can be of any crystallized species or even edible oil.

2.2 Introduction

Polymers such as methylcellulose (MC) and gelatin (GTA) have been shown to form hydrogels when dispersed in water. When MC is dissolved in water, “cage-like” structures are formed which surround the hydrophobic methoxyl groups, causing the MC to become soluble in water and imparting consistency to the solution (Nasatto et al., 2015; Sarkar, 1995). Once heated, these structures distort and break to expose the hydrophobic regions, inducing the formation of aggregates (Haque & Morris, 1993). Subsequently, these aggregates phase separate which is accompanied by gelation (Kobayashi, Huang, & Lodge, 1999). In addition, as MC forms a gel upon heating, it can act as a good coating material in frying applications because it hinders water evaporating and oil from entering, thus the oil uptake is reduced (Mellema, 2003).

Likewise, GTA is one of the most versatile and utilized gelling agents in food application due to its special texture and the melt-in-mouth perception (Haug, Draget, & Smidsrod, 2004). When dispersed in water at a concentration up to 300 g/L, GTA forms low viscosity solutions at temperatures above 40°C. Upon cooling to room temperature, the solution turns to a clear, rubbery, and thermo-reversible gel. Formation of such a gel is the result of overlapping polymers, which leads to the formation of junction zones and ultimately to the formation of a three-dimensional branched network (Gilsenan & Ross-Murphy, 2000). This unique property of GTA expands its application in food industry not only as gelling agent but also as film forming agent to produce biodegradable packaging materials (Hanani, Roos, & Kerry, 2012; Rhim & Ng, 2007).

The functionality of these hydrophilic polymers cannot be exploited in lipophilic systems (edible oil) because they are not soluble in non-polar solvents. Most hydrophilic polymers should be effectively hydrated or un-coiled to be functional (Haug et al., 2004; Laaman, 2011; Patel & Dewettinck, 2016).

Polymers containing a hydrophilic and hydrophobic part, such as MC and GTA, have surface active properties and can stabilize emulsions (Dickinson, 2009; Nasatto et al., 2015). Therefore, the surface activity of these two polymers is a potential route for inclusion into hydrophobic solvents (Dickinson, 2009; Hayakawa et al., 1997). Numerous techniques have been employed to incorporate hydrophilic polymers as co-structurant in lipophilic media. The techniques consist of emulsion-templated approach (Patel, Cludts, Bin Sintang, Lesaffer, et al., 2014; Patel, Cludts, Bin Sintang, Lewille, et al., 2014; Patel, Rajarethinem, et al., 2015; Romoscanu & Mezzenga, 2006; Tavernier, Patel, et al., 2017), foam-templated approach (Patel, Schatteman, Lesaffer, et al., 2013), and solvent-exchange approach (de Vries et al., 2015), which all successfully exploit a polymer network as a structuring building block for edible oil. In the emulsion-templated approach, an oil-in-water emulsion is dried, leaving only the hydrated polymer to structure the oil. The foam-templated approach on the other hand relies on the ability of hydrophilic polymers to stabilize air-droplets. Eventually, the dried foam has an ability to absorb liquid oil (oil sorption) (Patel, Schatteman, Lesaffer, et al., 2013). In the solvent-exchange approach, a protein hydrogel was first prepared and the encased solvent (water) was exchange with liquid oil, using acetone or tetrahydrofuran as intermediate solvent (de Vries et al., 2015; de Vries, Wesseling, et al., 2017). However, the use of organic solvents limits the application of the solvent exchange approach in foods.

The success of the emulsion-templated approach to structure liquid oil, opens potential for further development. Yet, the polymer network is prone to collapse during high-temperature drying and shearing process of the dried emulsion, which limits its functionality to structure liquid oil. Therefore, based on the surface-active properties of hydrophilic polymers and similar hypothesis as in emulsion-templated approach, we hypothesized the formation of polymer protective layer at the interface of oil-in-water emulsion (Dickinson, 2009; Hayakawa et al., 1997). Contrary to the emulsion-templated approach, we introduced a crystallized fat instead of oil, which can become

the carrier for the hydrophilic polymers. The oil-droplets crystallize when the emulsion is cooled, which causes emulsion destabilization and forms a cream layer. The cream layer consists of crystallized oil-droplets coated with hydrophilic polymer, forming the protective layer. This approach is also adopted from the preparation of solid lipid nano/microparticles (SLN) through micronization(microfluidizer) process (Kim & Vanapalli, 2013; Letourneau, Vigneau, Gonus, & Fages, 2005).

The preparation involved the adsorption and fixation of the hydrophilic polymer at the water-oil interface prior to drying. Consequently, the fat acted as carrier for the hydrophilic polymers to be introduced into the sunflower oil. Nonetheless, this chapter is a proof-of-concept that introduces the innovative approach to carry hydrophilic polynmer to sunflower oil. Therefore, the internal fat phase (fat capsules) can be explored beyond FHRO and might possible to incorporate oil to reduce the solid fat. However, changing the internal phase will affect the crystallization process which might influence the morphology of fat capsules. Thus, proper equipment such as temperature controlled microfluidizer is necessary for such development to fabricate discrete spherical particles. Oleogels made from polymer coated fat crystals were characterized rheologically and morphologically to study the effectiveness of the technique. The outcomes of this study are potentially beneficial as an alternative for modifying the rheological properties of fat-based system through jamming (volume) effect, which is a form of physical approach. The creation of the oleogel system comprising of fat crystals network as the main structurant and polymer as the co-structurant provides alternative to the current approach of using emulsifier and interesterification to modify the physical behavior of fat structure.

2.3 Materials and methods

2.3.1 Materials

The fully hydrogenated rapeseed oil FHRO (PA6111), and sunflower oil were supplied by Vandemoortele Lipids N.V., Belgium. The methylcellulose (MC), 1500 cPs, was obtained as free gift from HARKE FoodTec, Germany. Gelatin type A (GTA) was obtained from PB Gelatins GmbH, Belgium.

2.3.2 Preparation of fat capsules

The aqueous polymer solutions were prepared by dispersing 2.0 wt% polymer in distilled water and heated to 70°C. The FHRO was heated to 90°C to erase the crystal history and to ease the emulsification process. The molten FHRO was poured into the polymer solution in a 30/70 (O/W) ratio, respectively. Subsequently, the mixtures were emulsified at 70°C with a high-energy dispersing unit (Ultraturrax, IKA-Werke GmbH & Co. KG, Germany) at 11000 rpm for 10 minutes. Eventually, the O/W emulsions were poured into a cold water solution of at least 10 times the volume of the emulsion. This promotes creaming of the polymer coated fat crystals to the surface. Then, the water solution containing the emulsion was stored inside a fridge for 24 hours to induce creaming. After 24 hours, the coated fat crystals formed a thin layer on top of the cold water solution, which was scraped off manually and placed on a drying tray. Finally, the coated fat crystals were dried at 33°C for 12 hours to evaporate the remaining water. The dried coated fat crystals turned into a powder form existing out of fat capsules. The moisture content (%) of fat capsules measured using Karl Fischer is as followed; MC-based = 0.92 ±0.04, GTA-based = 1.08 ±0.09. The capsules were stored at 5°C.

2.3.3 Preparation of oleogels

Oleogels were prepared by dissolving 12 wt% fat capsules in sunflower oil (SFO) at 90°C (12 wt% is the minimum gelling concentration of FHRO in sunflower oil (Figure A1). The oleogel containing only FHRO at 12 wt% concentration acted as a reference. During preparation, a magnetic stirrer (Model EM3300T, Labotech Inc., Germany) continuously stirred the solution for 20 minutes. This process assists in distributing the adsorbed polymer evenly in the solution prior to crystallization of fat. The samples were transferred to a plastic container and allowed to cool at room temperature for 10 minutes. Finally, the oleogels were transferred to a fridge at 5°C.

2.3.4 Thermal behavior

The thermal behavior of the fat capsules and oleogels was analyzed using Q1000 DSC (TA instruments). The DSC was calibrated with indium (TA Instruments), azobenzene (Sigma–Aldrich,Bornem, Belgium) and undecane (Acros organics, Geel, Belgium) prior to analysis.

At least 5 mg of the capsules and oleogels were transferred into an empty DSC pan , which was hermetically sealed. The thermal behavior of the capsules was analyzed by investigating their melting and crystallization behavior. The capsules were heated from 20°C to 90°C at a heating rate of 5°C/min, then crystallized at a cooling rate of 10°C/min to 5°C. The thermal behavior of the oleogels was analyzed by heating the oleogels to 90°C to eliminate any crystals memory. Then, the samples were crystallized by cooling to 5°C at a cooling rate of 10°C/min. At 5°C, the samples were allowed to crystallize for three hours (isothermal crystallization) before being heated again to 90°C at a heating rate of 5°C/min. The pans were immediately transferred into a fridge and stabilized for at least one week.

After the stabilization period, the pans were subjected to melting analysis. The melting analysis was performed by heating the pan from 5°C to 90°C, at a heating rate of 5°C/min.

2.3.5 Small amplitude oscillatory stress

All the analysis were conducted using the rheometer AR2000ex (TA Instruments, New Castle, DE) with the Advantage application software. The analyses were conducted triplicate for the same oleogel of three different independent oleogels.

2.3.5.1 Oscillatory temperature ramp and time sweep

Oscillatory temperature ramps and time sweeps (frequency: 1Hz, strain (%): 0.01) were performed on the oleogels by using the starch rotor cell geometry (gap = 5500 µm). Thirty (30) gram of molten sample was transferred into the geometry and conditioned at 90°C for 10 minutes before cooling to 5°C with a cooling rate of 10°C/min. The samples were then allowed to set inside the geometry while measuring the complex modulus (|G*|) and phase angle/delta (δ) as a function of temperature and time at 5°C (for three hours).

2.3.5.2 Amplitude stress sweep

Amplitude stress sweep (frequency of 1 Hz; oscillatory stress: 1 to 1000 Pa, at 5°C) was performed on the oleogels immediately after the end of time sweep, using starch rotor cell geometry. This analysis serves to determine the linear viscoelastic region (LVR) of the oleogels.

2.3.6 Optical light microscopy

The crystal morphology of the oleogels was visualized after one-week stabilization at 5°C. After putting the sample on a glass microscope slide and covered by a cover slit, the samples were then placed on the microscope stage. The microscope stage was regulated at 10°C (Temperature control stage; Linkam T95 System Controller (Linkam Scientific InstrumentLtd., Surrey, UK)) to avoid condensation during visualization. Light microscopy, a Leica DM2500 microscope (Leica Microsystems, Belgium) equipped with a color camera Leica MC170 HD was used.

2.3.7 Scanning electron microscopy

To allow visualization with cryo-SEM, sample preparation was required to reveal the internal fat crystal network. The oil from the network structure was removed by using the adapted method of Maleky and co-workers (Maleky et al., 2012).The oleogels were suspended in excess ethanol to remove liquid oil from the structure (12 hours). The oleogels were then filtered to remove the ethanol and mounted on an aluminum stub. The samples were then quickly frozen in slushed nitrogen (-210°C), fractured, sputter-coated with platinum and observed with a Jeol JSM-7100 F scanning electron microscope (Jeol Europe) B.V., Zaventem, Belgium).

2.3.8 Confocal laser scanning microscopy

The location of the polymers was confirmed using CLSM. Two different fluorescent dyes were selected based on their affinity for the polymers, fluorescein isothiocyanate (FITC, (GTA)) and rhodamine 6G (MC) (Sigma-Aldrich, Bornem, Belgium). The dye (50-200µg/ml) was dissolved in the polymer solution which was used for the preparation of fat capsules. The stained fat capsules were subsequently used to prepare the oleogels. Small amounts of fat capsules and oleogels were smeared onto a glass slide and covered with a cover slid.

The visualization was conducted using Nikon A1R confocal laser scanning microscope (Nikon; Tokyo, Japan) with a 60X (oil lens) [Plan Apo VC 60x Oil DIC N2; Nikon Instruments, Paris, France] Nikon Instruments. For samples labelled with FITC, the laser with wavelength 488 nm at 5% power was used to excite the fluorescent dye and the emission was measured with a 525/50 filter with green channel. Whereas, the samples labelled with rhodamine 6G, the laser with wavelength 514 nm at 6.7% power

was used to excite the fluorescent dye and the emission was measured with a 540/30 filter with green channel. The scanner zoom was 2 with speed of 1frame/second. The resultant images were analyzed using imageJ software.

2.3.9 Powder x-ray diffraction

The molecular organization of the fat capsules and oleogels was determined by XRD spectroscopy using a D8 Advance Diffractometer (Bruker, Germany) equipped with an X-ray generator Kristalloflex K780 (Bruker, Germany) (Cu, λ=1.54178Å, 40kV, 30mA), and a temperature control unit (TCU110system, Anton Paar, Austria) connected to a water bath. This system allows adjustments of the temperature of the thermostated diffractometer chamber TTK 540 (Anton Paar, Graz, Austria). Both small angle X-ray diffraction (SAXD) and wide-angle X-ray diffraction (WAXD) were recorded at 5°C.

2.3.10 Statistical analysis

The experimental data are expressed as means ± standard deviation of three repetitions. The statistical analyses were performed using SPSS version 24. One-way ANOVA to find the significant different ($P<0.01$ and $P<0.05$) of more than two variables and independent T-test to evaluate the significant different ($P<0.01$ and $P<0.05$) of two variables were applied depending on the compliance of data to a normal distribution.

2.4 Results and discussion

2.4.1 Preparation and characterization of fat capsules

2.4.1.1 Fabrication technique and morphology of fat capsules

Emulsification technique in combination with drying technique (Figure 2.2) was developed to introduce hydrophilic polymers as co-structurants into a lipophilic medium. Based on the ability of hydrophilic polymers to adsorb at an oil-water interface, emulsion droplets were chosen as vehicle for polymer introduction (Dickinson, 2009; Haug et al., 2004). The chosen concentration of the polymer solution (2.0 wt%) and water-to-oil ratio (70:30) were decided based on a few considerations. The concentration of the polymer was chosen as such to achieve optimal delivery of the polymers to sunflower oil in the form of adsorbed layer at the interface of fat capsules. The adsorption properties of methylcellulose and gelatin at oil-water and air-water interfaces are based on their ability to saturate the interfaces (McClements,

2005). The interfacial saturation with these polymers can be measured through surface activity and surface load, which were studied quantitatively by Arboleya and Wilde (2005), Surh *et.al* (2006), and Zhang *et.al* (2017) using Wilhelmy plate method. These studies reported the saturation of polymers, MC and GTA, at the oil-water interface at approximately 0.01 %(w/v) of saturation point (Arboleya & Wilde, 2005; Zhang, Yang, Liu, & Li, 2017). Additionally, Zhang and co-workers (2017) reported that increased polymers (emulsifiers) concentration in water phase can increase the number of effective contact polymer groups and lower the interfacial tension (Zhang et al., 2017). Moreover, Dickinson (2009) explained the capability of polymers to form a stabilizing (protective) layer at the interface at polymer concentration exceeding the saturation. Therefore, 2 wt% of polymer was selected to prepare the aqueous solution which translated to 1.4 wt% of polymer concentration during emulsification (fabrication). This concentration is deemed sufficient to ensure complete adsorption of polymer at the interface, as it is above the saturation point. However, one cannot simply choose a higher concentration, especially for methylcellulose. At considerably higher concentration, methylcellulose (1500 cPs) becomes difficult to dissolve and hydrated with water during the preparation of the aqueous solution. This limitation was observed in other technique of oil structuring involving emulsion-templated approach (Patel, Cludts, Bin Sintang, Lesaffer, et al., 2014).

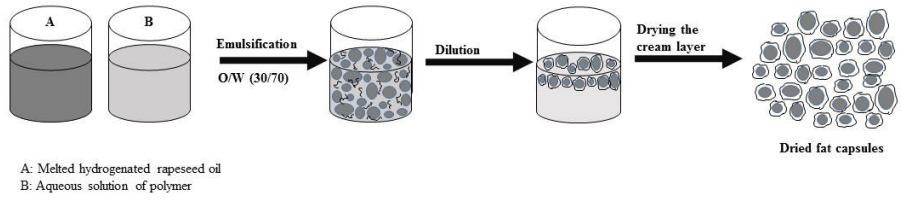

Figure 2.2 Processing steps to preparation of fat capsules.

Secondly, the chosen ratio of 70:30 (W:O) was based on the preliminary screening study of different ratios using palm stearin as the dispersed phase (Patel, 2017). It was shown that the different in water-to-oil ratios in the fat capsules preparation did not influence the rheological properties. The rheological properties of fat capsules prepared at 70:30 and 90:10 (water:oil) had almost similar rheological properties. Since the aim of this study was to use crystallized fat droplets as carrier for hydrophilic

polymers, the 70:30 (water:oil) was chosen to increase the rate of delivery. Additionally, the efficient coverage of the interface by polymer was obtained with such concentration. However, when the water to oil ratio was increased to 40:60, the palm stearin capsules lost its functionality (Patel, 2017).

The fabrication process of the fat capsules was adapted based on the production process of fat (palm stearin) micro/nano particles through micronization (Kim & Vanapalli, 2013; Letourneau et al., 2005; Munuklu & Jansens, 2007). As illustrated in Figure 2.2, melt homogenization (emulsification) was performed to fabricate spherical fat capsules (Figure 2.3). The GTA-based capsules had smaller droplet size than the MC-based capsules. The difference in droplet (capsule) size can be explained by the higher surface activity of GTA than MC, affecting the kinetic of adsorption to the interface thus the droplet size (Arboleya & Wilde, 2005; Dickinson, 2003, 2009; Zhang et al., 2017). Less time is required for GTA or protein to adsorb at the interface is than for MC thus, GTA quickly reduces the interfacial tension and facilitate the droplets disruption (into appreciably smaller droplets) (McClements, 2004). The difference is associated with the different in molecular weight between GTA and MC, which the latter has higher molecular weight than the former. Dickinson (2009 and 2003) explained that the higher molecular weight of polymer-based stabilizer results in coarse or bigger droplets emulsion than emulsion prepared using low molecular weight surfactant and proteins.

Simultaneous to the emulsification, the hydrophilic polymers adsorbed at the interface of water-and-oil. It was reported by Floury and co-workers (Floury, Desrumaux, Axelos, & Legrand, 2003) that the adsorption of hydrophilic polymers such as MC and GTA to the interface involves the diffusion-mediated and convective material transporting from the bulk (Dickinson, 2009). The adsorption at the water-oil interface creates a protective layer of polymer (Dickinson, 2009; Dickinson, Stainsby, & Wilson, 1985; Hayakawa et al., 1997). In the continuous phase, the polymers exist in coiled conformation, but after adsorption the polymer backbone starts to uncoil (Arboleya & Wilde, 2005; Perez, Carrera-Sanchez, Rodriguez-Patino, & Pilosof, 2007).

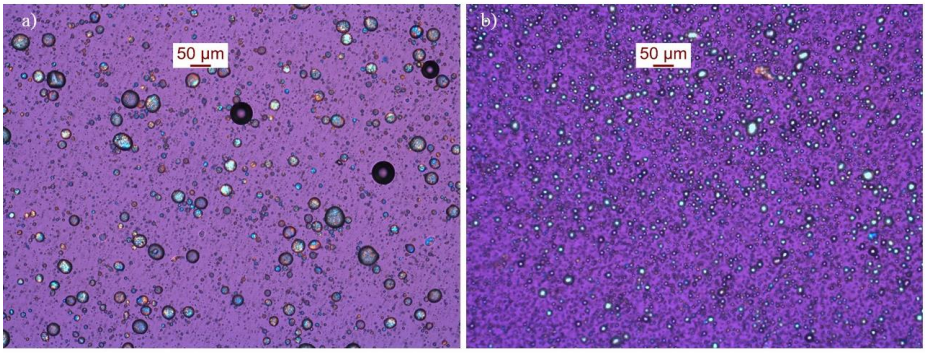

Figure 2.3 Polarized light microscopy of the fat capsules coated with Methylcellulose (MC) (a) and Gelatin (GTA) (b).

The formation of a protective polymer layer at the water-oil interface was visualized using SEM and CLSM microscopy. Figure 2.4 exhibits the electron microscope images of fat capsules coated with MC (Figure 2.4a and 2.4c) and GTA (Figure 2.4b and 2.4d). Generally, the fat capsules coated with MC and GTA had a spherical shape and were polydisperse in droplet size. All the fat capsules displayed rough surface.

In the fabrication process of fat microparticles, the core material (FHRO) crystallized by pouring the emulsion in cold water. Thus, a rapid crystallization of FHRO was achieved, allowing the fixation of a MC or GTA layer at the interface. Moreover, diluting the emulsion with cold water removed the non-adsorbed polymers from the continuous phase. Kim and Vanapalli (2013) tested different temperatures of exiting capillary (outlet) of microfluidizer, which appeared to influence the products' morphology in the production of fat microparticles. In our fabrication process, diluting the O/W emulsion in cold water (5°C) triggered the crystallization of FHRO and possibly prevented partial coalescence, wherein the temperature of water in our study is analogous to the temperature of exiting capillary. As shown in Figure 2.4, no perceptible coalescence was observed between the capsules in both preparation.

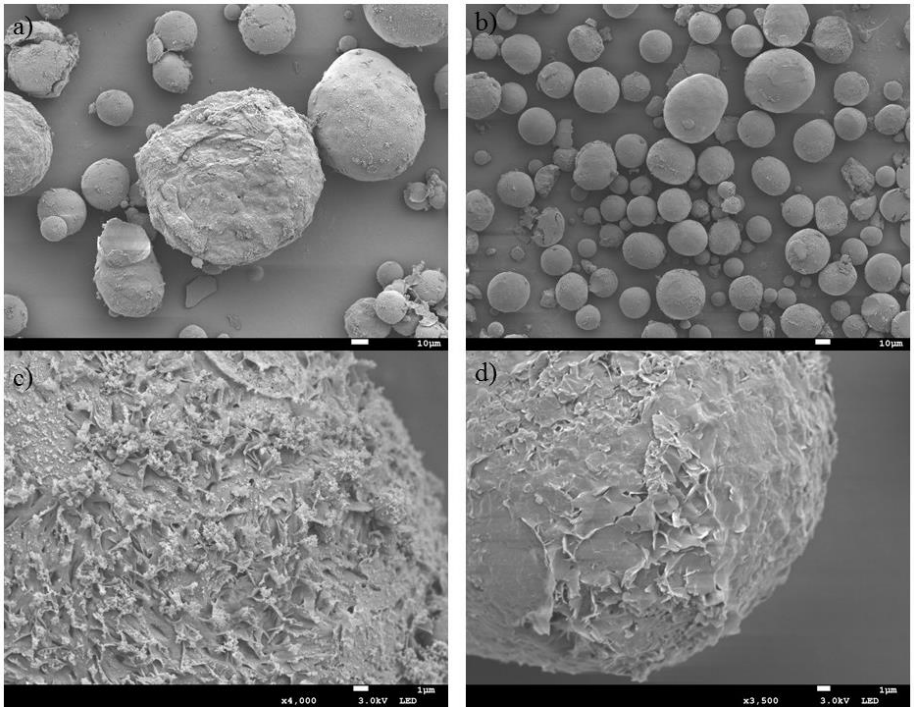

Figure 2.4 The cryo-SEM images of fat capsules coated with Methylcellulose (MC) (a and c) and Gelatin (GTA) (b and d). The scale bar a and b is 10µm while c and d is 1µm.

The adsorption of MC and GTA at the interface was confirmed using the CLSM technique. It is important to confirm the presence and location of a polymer to evaluate the effectiveness of this new approach. Figure 2.5 clearly shows the location of polymer layer at the surface, encasing FHRO. The confocal images in Figure 2.5 (z-stack) and Figure 2.6 show surfaces of fat capsules illuminated in green, which are clearer for GTA-based than for MC-based fat capsules. For clarity, the brightfield images are provided in Figure 2.5 and Figure 2.6. The green surface of fat capsules came from the fluorescence dyes (FITC and Rhodamine 6G), attached to the respective polymers. The attachment of FITC to gelatin or proteins was based on the interaction of fluorescein, particularly isothiocyanate, to the N-terminal end and/or cysteine group through non-covalent interaction (Sameiro & Goncalves, 2009; Toseland, 2013; van de Velde, Weinbreck, Edelman, van der Linden, & Tromp, 2003). The confocal visualization for MC-based fat capsules was not easy to perform, as most of fluorescent labelling procedure for polysaccharides requires covalent interaction which might

influences the conformation of MC. Nonetheless, rhodamine 6G is water soluble and able to non-covalently attach to MC during hydration which also allows the dye to accumulate with the MC (van de Velde et al., 2003). It is important to stress that the CLSM images obtained are only for localization and not for quantification, which the latter requires specific stoichiometry calculation and covalent labelling between dye and subject.

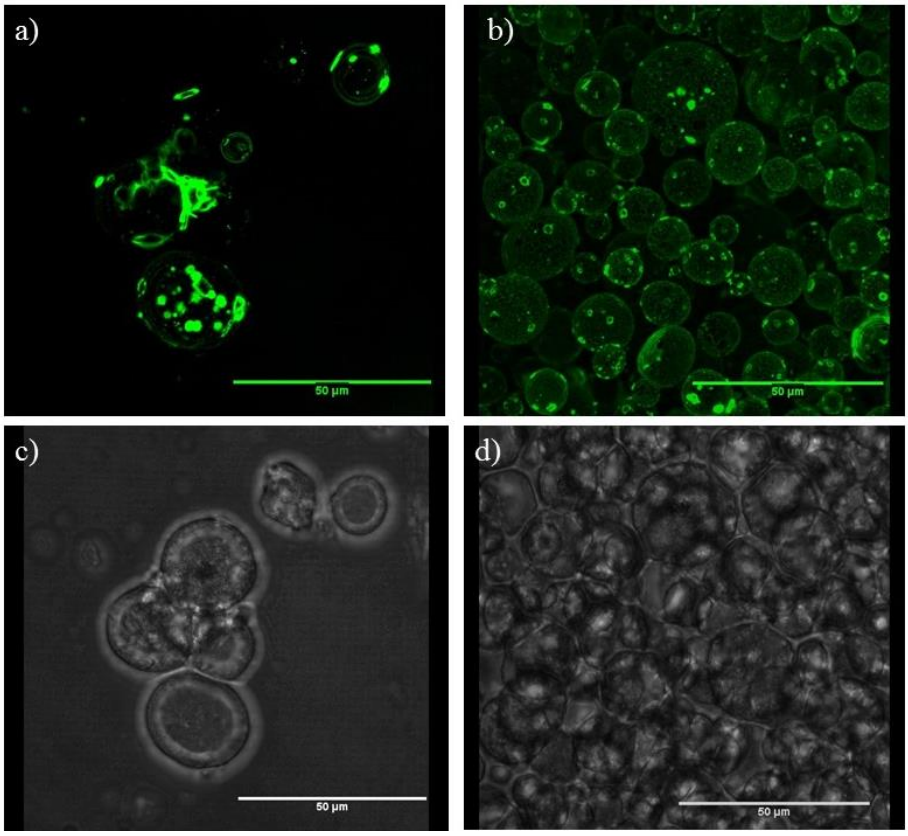

Figure 2.5 The three-dimensional reconstruction (z-stack) from CLSM images of fat capsules coated with Methylcellulose (MC) (a and c) and Gelatin (GTA) (b and d). The a and b images represent the flurescent, while c and d images represent the brightfield. The scale bar is 50 µm.

In Figure 2.5, the three-dimensional images, especially in GTA-based capsules, illustrates a discrete spherical shape of fat capsules with the internal part (core)

appears dark (unlabeled FHRO), as also shown in Figure 2.6. Figure 2.6 represents the images taken at one plane, which demonstrate the adsorption of the polymers at the interface (Figure 2.6a and 2.6b). The CSLM imaging confirms that the fat capsules retained a spherical shape probably without coalescence, despite being concentrated in the cream layer. This observation suggests that crystallization of FHRO prevents the droplets to coalesce, which is also due to the adsorption of polymer at the interface, imparting steric stabilization (repulsion).

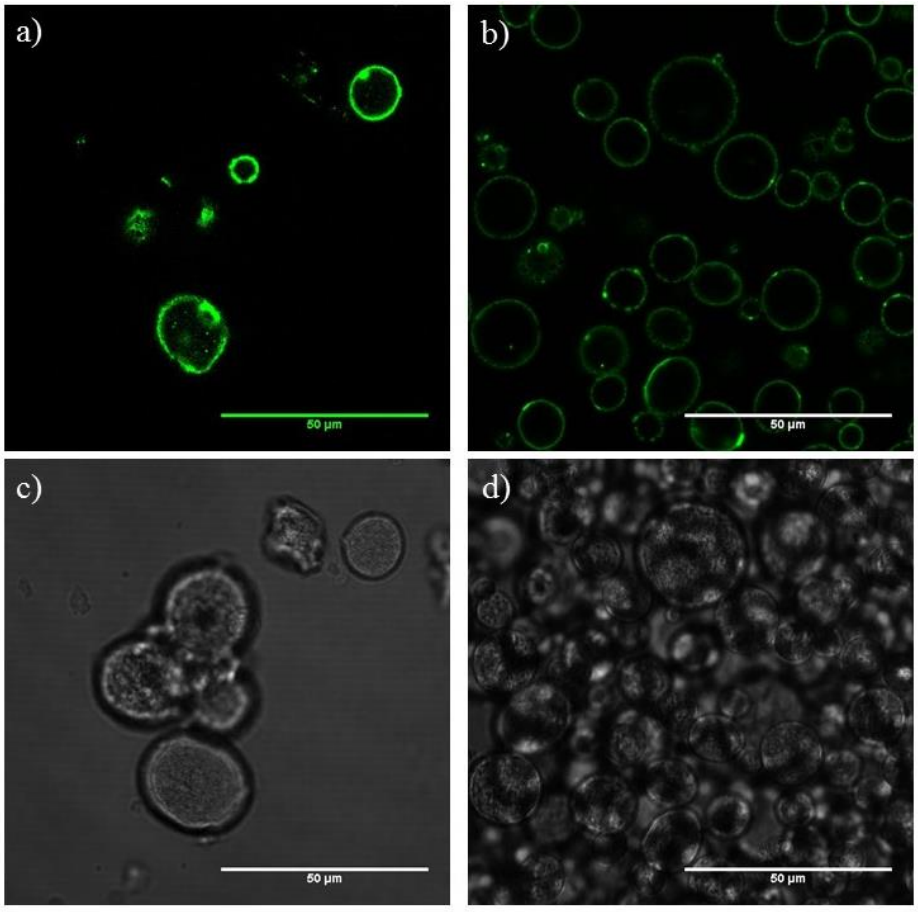

Figure 2.6 The CLSM images (from one plane) of fat capsules coated with Methylcellulose (MC) (a and c) and Gelatin (GTA) (b and d). The a and b images represent the fluorescent, while c and d images represent the brightfield. The scale bar is 50 µm.

2.4.1.2 Thermal behavior of resultant fat capsules

To elucidate the effect of the fabrication steps and the presence of the hydrocolloids MC and GTA on the crystallization of FHRO, DSC analysis was conducted. The MC and GTA can act as impurities, which could influence the crystallization of FHRO (Bayes-Garcia et al., 2015; Coupland, 2002; Sato et al., 2013). GTA and MC may influence the nucleation step of FHRO by providing a surface for nucleation. In a study by Rogers and co-workers, gelatin provided a template that accelerated the nucleation of high melting fraction of fat (Rogers, Wright, & Marangoni, 2005).

The melting behavior of fat capsules is shown in Figure 2.7a and relevant parameters are listed in Table 2.1. The fabrication steps and the addition of the MC or GTA do not seem to influence the crystallization of coated FHRO, which the melting temperature and enthalpy were not significantly different. Upon cooling, the FHRO coated with MC and GTA crystallized at the same temperature as pure FHRO (Table 2.1 and Figure 2.7b). Though the onset crystallization was significantly different between the samples, the change can be considered minimal as the difference in crystallization enthalpy was not significant. Hence, the results imply that the FHRO crystallized independently without being influence by the external factors (fabrication steps, MC, and GTA).

Table 2.1 The peak melting temperature (T_{mp}), melting enthalpy (H_{mp}), onset crystallization temperature (T_{on}), and crystallization enthalpy (H_{cry}) of the raw fully hydrogenated rapeseed oil (FHRO) and the Methylcellulose (MC) and Gelatin (GTA) capsules (N=3, of independent samples).

Samples	T_{mp}(°C)	H_{mp} (J/g)	T_{on} (°C)	H_{cry} (J/g)
FHRO	61.20±0.97[a]	135.83±1.99[a]	57.40±0.02[a]	151.70±2.10[a]
MC-capsule	60.39±1.19[a]	136.50±4.87[a]	58.03±0.03[c]	153.70±3.30[a]
GTA-capsule	59.74±0.09[a]	133.47±4.76[a]	57.72±0.02[b]	156.84±1.85[a]

Values indicated with the same letter in the same column are not significantly different (P < 0.01)

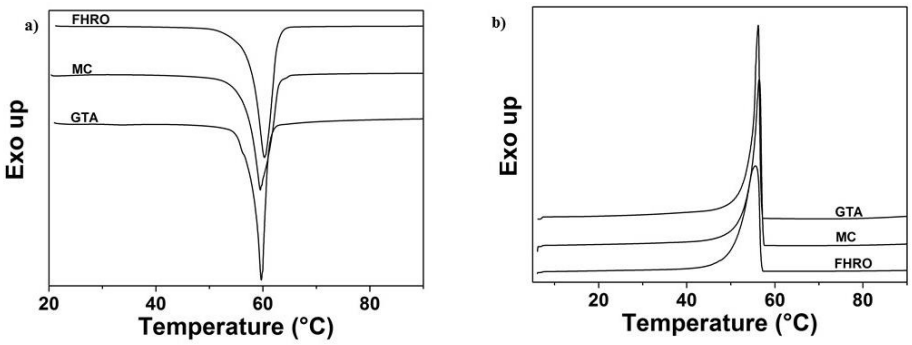

Figure 2.7 Melting profile (a) and crystallization profile (b) of fat capsules coated with Methylcellulose (MC) and Gelatin (GTA). For comparison, the melting and crystallization profiles of Fully hydrogenated rapeseed oil (FHRO) were also measured.

This outcome contrasts with the findings of Rogers and co-workers (2005), where gelatin did act as a template for crystallization of fat. To explain this, the kinetics of crystallization of FHRO and melt homogenization process have to be considered. In a study executed by Vanapalli and co-workers, no flocculation of n-hexadecane emulsion was observed due to sharp melting and crystallization peak, which indirectly resisted flocculation of droplets (Vanapalli, Palanuwech, & Coupland, 2002a, 2002b). Like our system, the inability of the polymers to influence crystallization of FHRO may be due to the fast crystallization of FHRO. In addition, the concentration of the impurities (MC and GTA) and the degree of undercooling, could also influence the magnitude of the effect that these impurities can exert on the crystallization of FHRO (Sato et al., 2013). Furthermore, for the impurities to act as a seed that influences the crystallization, there is a need for structural similarity between the seed and the fat molecules (Sato et al., 2013; Smith et al., 2011).

2.4.1.3 Molecular organization of fat capsules

The effect of the fabrication steps and the addition of MC and GTA on the molecular organization of FHRO was investigated using powder-XRD. The crystallization (polymorphic form) of solid fats in O/W emulsion droplets influences the stability, rheology and appearance of the emulsion (Sato & Ueno, 2011). Therefore, the polymorphic forms of the resultant fat capsules should be investigated since

incompletely crystallized FHRO may destabilize the emulsion droplets during storage due to isothermal crystallization (Coupland, 2002; Kim & Vanapalli, 2013).

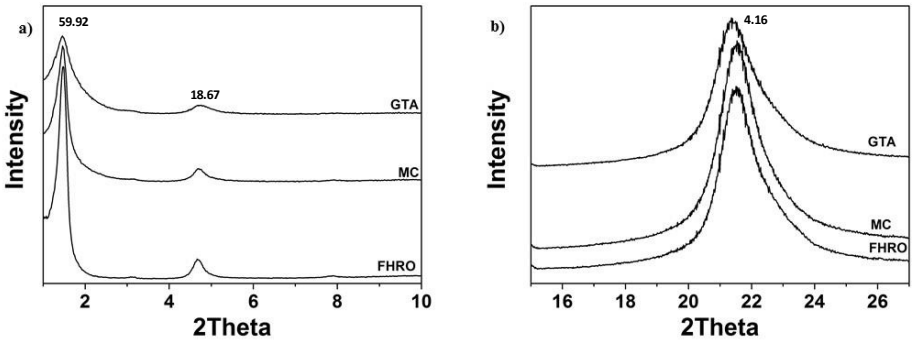

Figure 2.8 WAXD (a) and SAXD (b) of Fully hydrogenated rapeseed oil (FHRO), Methylcellulose-based (MC), and Gelatin-based (GTA) fat capsules measured at 5°C after at least one week stabilization at 5°C.

Figure 2.8 shows the diffraction patterns obtained at small angles (SAXD) and wide angles (WAXD). In SAXD, pure FHRO produced a sharper peak than the fat capsules coated with MC and GTA. In the WAXD region, the α-polymorph (4.16Å) was detected for all samples (Munuklu & Jansens, 2007). In general, it can be concluded that the fat capsules produced identical diffraction patterns compared to FHRO. Hence, we propose that the fabrication process and the presence of MC and GTA did not have a substantial effect to the molecular organization of FHRO. These diffraction patterns also agree with the thermal behavior discussed in part 2.4.1.2. The information from the diffraction study provides interesting insight particularly in the similarity of α-polymorph between FHRO (reference) and the fat capsules. For drug carrier, it is recommended to have loose packing of core material such as α- or β'-polymorph to efficiently encapsulate drugs (Scalia et al., 2015).

The production of stable fat microparticles using an adapted approach from the micronization production of fat microparticles was successful. In contrast to conventional fat microparticles, our fabrication process produced fat microparticles coated with either MC or GTA. The presence of MC or GTA at the surface of fat microparticles could broaden their potential application The fabricated fat capsules characterized in the previous section have analogous morphology and properties as

solid lipid microparticles. From the perspective of solid lipid microparticles, the characterization studies presented above showed the potential of fat capsules to deliver functional ingredients for food or drugs for pharmaceutical applications. This mainly due to the internal fat phase crystallized independently with less influence from the fabrication process and the polymers. Scalia and co-workers explained that the fat morphology and polymorphism have a great influence on the efficiency of solid lipid microparticles as a delivery agent (Scalia et al., 2015).

In the following section, we prepared oleogels using the fat capsules to study their behavior as an oil structuring agent. Our hypothesis is that both the aqueous polymer and the FHRO will contribute to oil structuring, in which the former acts as a co-structurant.

2.4.2 Oil structuring properties of fat capsules in sunflower oil

Oleogels were prepared using the fat capsules as structurants. Oleogels containing solely FHRO functioned as reference. Figure 2.9 illustrates the gelation approach involving the fat crystals as the main structurant with polymer networks as the co-structurant. The polymer network (sheet) consolidates the fat crystal network by inducing jamming.

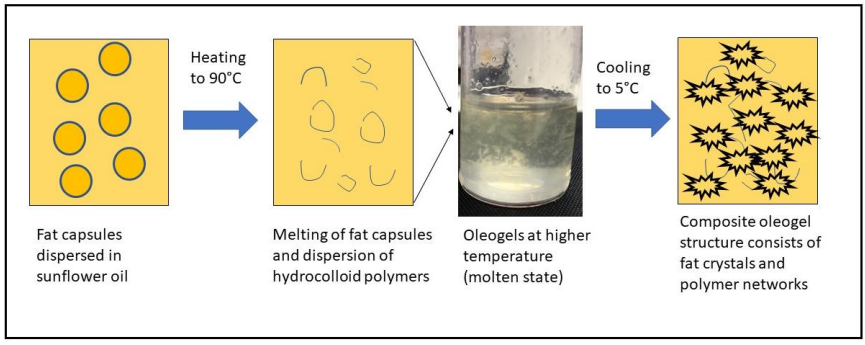

Figure 2.9 Schematic illustration of the formation of composite oleogel consists of fat crystals and polymers networks.

As the strands of the polymers remained dispersing in the oil, FHRO could crystallize before the strands settled to the bottom. This process allows simultaneous formation of oleogel structure based on the fat crystals and polymer network. The use of a

colloidal particle to induce jamming (volume) effect on fat crystals networks have recently investigated by Chauhan and co-workers (2017a and b) and Yoshikawa and co-workers (2015). It is wise to state that the application and approach employed in the preparation of oleogels are part of proof-of-concept study.

2.4.2.1 Rheological behavior of oleogels

To monitor the temperature- and time-dependent behavior of the oleogels (microstructural development over time at 5°C), an oscillatory temperature ramp and time sweep were performed. In this analysis, the |G*| and δ were followed-up in time during the isothermal period at 5°C to evaluate the effect of incorporating GTA and MC on the gelation behavior.

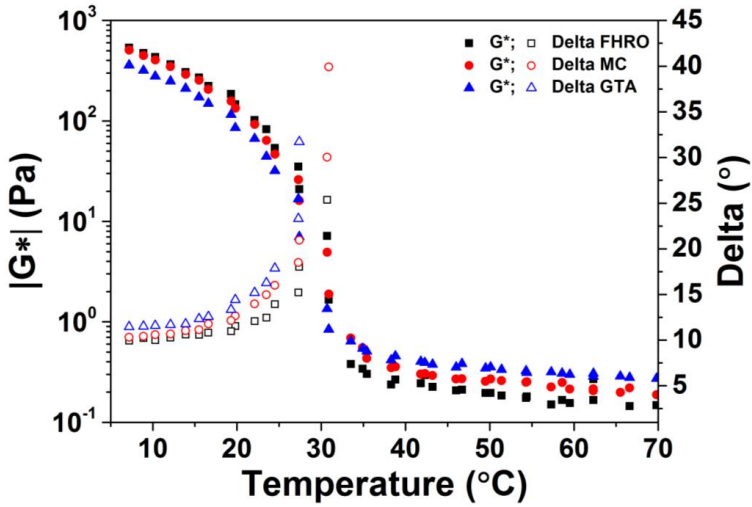

Figure 2.10 The evolution the complex modulus and delta as a function of temperature during cooling of the Fully hydrogenated rapeseed oil (FHRO), Methylcellulose (MC)-based, and Gelatin (GTA)-based oleogels with cooling rate of 10°C/min.

During the temperature ramp, the molten oleogels were cooled from 90°C to 5°C. At the transition temperature, the oleogels changed from liquid to solid (Figure 2.10). The gelation can be detected by the change in the |G*| and δ (Lupi, Greco, et al., 2016; Mezger, 2011). As shown in Figure 2.10, all the oleogels solidified during cooling as the δ reached 45° and below. All the oleogels showed an almost identical gelling point

(Table 2.2). Along with the decrease in the δ, all the oleogels showed an increase in the |G*|.

Table 2.2 The gelling temperature (T_{gel}) of the Fully hydrogenated rapeseed oil (FHRO), Methylcellulose (MC)-based, and Gelatin (GTA)-based oleogels during temperature ramp (N=3 of same oleogel)

Oleogels	T_{gel} (°C)
FHRO	30.77±0.76[a]
MC-capsules	31.10±0.30[a]
GTA-capsules	30.17±0.76[a]

Values indicated with the same letter are not significantly different P < 0.05

As shown in Figure 2.11, all the oleogels displayed a similar pattern in the evolution of |G*|, which indicates a similar microstructural development during a time sweep at 5°C. The crystallization of fat crystals is therefore independent on the presence of the polymers. The oleogels prepared with the fat capsules, which thus also contain the polymers, had the highest |G*| values. Hence, the polymers presumably further boost microstructural development. Among the polymers, the MC was more effective than GTA in terms of microstructural development kinetics and |G*| value, which could be related to the different molecular structures of polymers. The MC oleogel outperformed the other two oleogels from the start of time sweep. The GTA oleogel clearly deviated from the FHRO oleogel only at the end of the measurement period (Figure 2.11 and Table 2.3). Addition of colloidal particles such as talc (Yoshikawa et al., 2015) or silica (Chauhan et al., 2017a) into fat crystal networks was found to influence the rheological behavior. Like the result obtained here, the presence of the polymers may act as a colloidal particle (aggregate) that boost the microstructural development measured as |G*|.

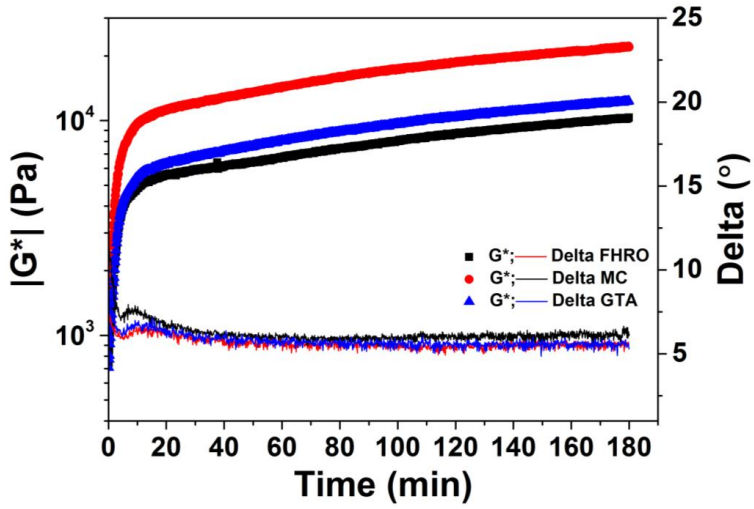

Figure 2.11. The evolution in complex modulus of the Fully hydrogenated rapeseed oil (FHRO), Methylcellulose (MC)-based, and Gelatin (GTA)-based oleogels during a three-hour period at 5°C, immediately after cooling.

Table 2.3 The phase angle (δ) and complex modulus |G*| values of the Fully hydrogenated rapeseed oil (FHRO), Methylcellulose (MC)-based, and Gelatin (GTA)-based oleogels at the end of a three-hour time-sweep (N=3 of same oleogel).

Oleogels	δ (°)	|G*| (kPa)
FHRO	6.68±0.50 [b]	10.56±0.97[a]
MC-based	5.64±0.21[a]	26.15±3.59[b]
GTA-based	6.03±0.21[ab]	12.85±1.11[a]

Values indicated with the same letter in the same column are not significantly different at P < 0.01

The resistance of the oleogels towards oscillatory stress was investigated by means of amplitude sweep by subjecting the oleogels to increasing oscillatory stress. Within the linear-visco elastic region, the applied oscillatory stress does not influence the G′ and G" value (Doan et al., 2015; Mezger, 2011). However, at a threshold stress where the oleogel is unable to maintain its structural integrity, the G′ and G" values become dependent of the oscillatory stress. Figure 2.12 illustrates that the presence of the polymers improved the tolerance of the oleogels towards the oscillatory stress. The G'_{LVR} values of the oleogels containing polymer were found to be higher and the LVR was longer. Another important parameter to evaluate the performance of oleogels is the G′-G″ cross-over point, the stress at which G' becomes equal to G". The cross-over

point was higher in the MC-based followed by GTA-based, and the lowest observed in FHRO oleogel (Figure 2.12).

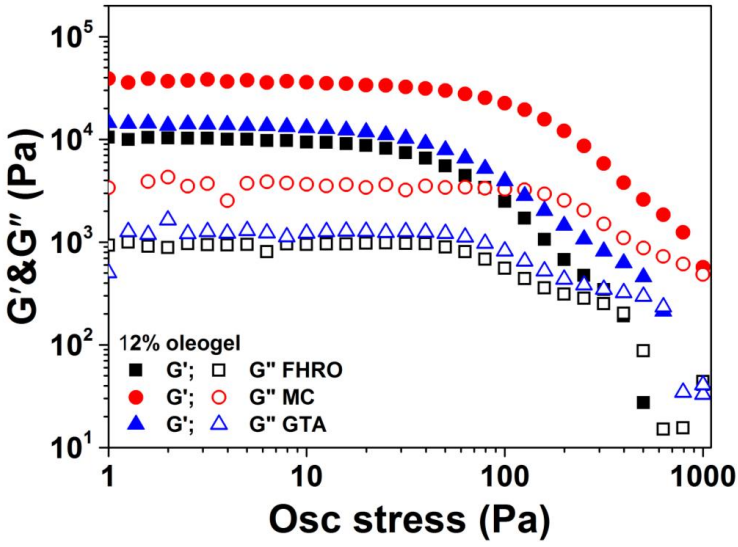

Figure 2.12. Amplitude sweeps at 5°C of the Fully hydrogenated rapeseed oil (FHRO), Methylcellulose (MC)-based, and Gelatin (GTA)-based oleogels measured at the end of three-hour time sweep.

In the oleogels prepared by using the emulsion-templated approach, MC showed to structure the liquid oil with improved rheological properties (Patel, Cludts, Bin Sintang, Lewille, et al., 2014). Likewise, the MC (1500 cPs) based oleogel in this study produced better structured oleogels with a higher storage modulus than the oleogels prepared with the emulsion-templated approach (Patel, 2015; Patel, Cludts, Bin Sintang, Lesaffer, et al., 2014; Patel, Rajarethinem, et al., 2015) and comparable moduli with the foam-templated approach oleogels of MC-based (Patel, Schatteman, Lesaffer, et al., 2013).

Whey protein oleogel (4.0 wt%) prepared by solvent-exchange (de Vries, Wesseling, et al., 2017) and gelatin-xanthan gum oleogel prepared from emulsion-templated approach (Patel, Rajarethinem, et al., 2015) had a lower storage modulus than our GTA-based oleogel. However, the addition of crystallized FHRO in oleogels might contribute to the high G' found in our study. Nevertheless, the difference in complex

modulus of the FHRO oleogels from the modulus of MC based and GTA based oleogels is attributed to the presence of MC and GTA in the respective oleogels.

The rheological properties of MC- and GTA-based oleogels can be ascribed to the presence of a co-structurant of a polymer network (a few networks randomly scattered). The polymer network acts similar to colloidal particles which fill the space between the crystal network (space-filling clusters) (Trappe & Sandkuhler, 2004). Chauhan and co-workers proposed the formation of composite oleogel networks consisting of fat crystals and silica particles which seemed to influence the rheology of oleogels (Chauhan et al., 2017a). Additionally, in the emulsion-templated approach, it was hypothesized that the strength of the MC film formed over the oil droplets was responsible for the rheological properties (Patel, Cludts, Bin Sintang, Lesaffer, et al., 2014; Patel, Rajarethinem, et al., 2015). This would explain the high G' of MC-based oleogel in our study. Whereas, gelatin networks show less contribution to the rheological properties of GTA-based oleogel. On the other hand, GTA-based oleogel did have improved rheological properties as compared to the FHRO oleogel. Therefore, our approach shows the supplementary effect of the polymers in structuring sunflower oil containing FHRO crystals. These results unequivocally show that hydrophilic polymers can function in hydrophobic solvent as co-structurants.

2.4.2.2 Molecular organization of oleogels

2.4.2.2.1 Thermal behavior

To understand the effect of the polymer on the crystallization of FHRO, the crystallization and melting behavior of the oleogels were investigated (Sato et al., 2013; Sato & Ueno, 2011). Litwinenko and co-workers investigated the effect of crystallization behavior on the mechanical properties of palm oil based shortening (Litwinenko et al., 2002). As was observed previously, the presence of MC and GTA influenced the rheological behavior of the oleogels. Understanding their thermal behavior helps to explain the improvement observed in their rheological properties, as mechanical properties are also related to thermal properties (Litwinenko et al., 2002).

Upon cooling, all the oleogels exhibited similar onset crystallization temperature (Table 2.4 and Figure 2.13a), similar to the gelation temperature (Table 2.2). However, there was a slight difference in the peak crystallization temperature, which was higher in

FHRO oleogel, yet insignificantly different than the other oleogels (Table 2.4). During melting, the oleogels showed a small crystallization peak prior to the main melting peak (Figure 2.13b and Table 2.5). This peak signifies the transition from metastable to stable polymorph, and was observed in all the oleogels. Although the FHRO oleogel exhibited significantly higher peak melting temperature, the peak shape and melting range remained the same in all the oleogels. Similarly, all the oleogels displayed slight variation in the melting enthalpy (Table 2.5).

Table 2.4 The onset temperature (T_{on}), peak temperature (T_{cry}), and enthalpy (H_{cry}) during crystallization of the Fully hydrogenated rapeseed oil (FHRO), Methylcellulose (MC)-based, and Gelatin (GTA)-based oleogels (N=3 of independent sample).

Oleogels	T_{on} (°C)	T_{cry} (°C)	H_{cry}(J/g)
FHRO	42.00±0.08^{a}	41.05±0.19^{a}	18.24±0.48^{a}
MC-capsule	42.15±0.22^{a}	40.64±0.05^{a}	18.80±0.39^{ab}
GTA-capsule	41.95±0.09^{a}	40.85±0.11^{a}	19.34±0.25^{b}

Values indicated with the same letter in the same column are significantly different only at P < 0.05

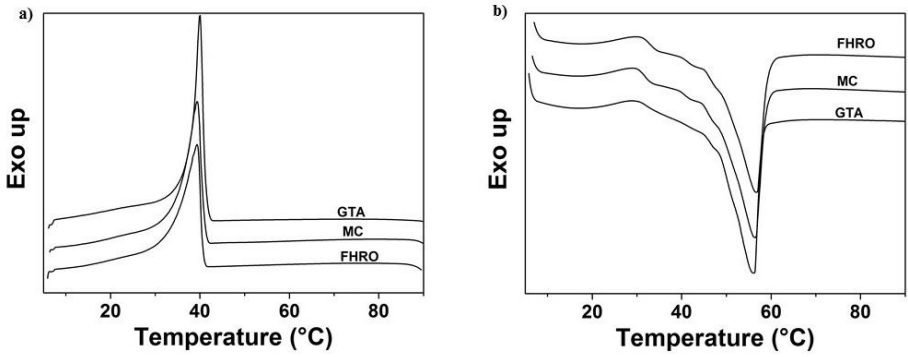

Figure 2.13 The cooling (a) and melting (b) thermogram of the Fully hydrogenated rapeseed oil (FHRO), Methylcellulose (MC)-based, and Gelatin (GTA)-based oleogels prepared using the FHRO and fat capsules.

A similar pattern was observed in the crystallization and melting behavior of the oleogels. The presence of MC and GTA did not promote nucleation of FHRO, which was also observed in the fat capsules discussed earlier. A study showed that the crystallization of tripalmitin was slightly hastened in the presence of silica due to volume effect (Chauhan et al., 2017a). The volume effect changed the lipid concentration as a part of the solution was occupied by a foreign component. The

polymers in this study however, did not induce any volume effect that might accelerate the onset of crystallization. Moreover, the shape of the crystallization peak and melting peak were identical in all the oleogels. It can be concluded that FHRO crystallized independently without any interference from the polymers, as was observed in the fat capsules.

The effect of storage on the melting behavior of the oleogels was again investigated after at least one-week stabilization at 5°C (Table 2.5). The results serve to elucidate the melting characteristic (polymorphs) of the oleogels as a function of storage time. Comparing the melting behavior between one-week (Figure 2.14) and three-hour storage (Figure 2.13b), there was change in the peak shape but not in the temperature range (Table 2.5). The temperature range of melting was identical between the stabilized and fresh (onset and offset of melting), which was also found similar in all the oleogels. The oleogels exhibited a recrystallization peak during melting of three-hour oleogels, which was absent in the stabilized oleogels. Hence, it can be assumed that the oleogels had achieved a stable state after one-week based on the melting profile. Additionally, there was no variation in the melting profile of the oleogels, as also seen in the previous analyses (Figure 2.13b). Thus, the thermal behavior of the oleogels provides strong evidence that the polymers did not affect the crystallization and polymorphic transition of FHRO.

Table 2.5 The peak melting temperatures (T_{mp}), melting enthalpy (H_{mp}), and offset melting temperature (T_{off}) of the Fully hydrogenated rapeseed oil (FHRO), Methylcellulose (MC)-based, and Gelatin (GTA)-based oleogels at different crystallization periods (N=3 of independent sample).

Oleogels	Three-hour			One-week		
	T_{mp} (°C)	H_{mp}(J/g)	T_{off} (°C)	T_{mp}(°C)	H_{mp}(J/g)	T_{off} (°C)
FHRO	56.63 ±0.04^{bA}	16.49 ±0.48^{aA}	63.62 ±0.54^{aA}	56.63 ±0.12^{aA}	19.99 ±1.33^{aB}	63.70 ±1.17^{aA}
MC_{cp}	56.31 ±0.14^{aA}	17.20 ±1.30^{aA}	63.36 ±0.89^{aA}	56.53 ±0.49^{aA}	19.04 ±1.81^{aA}	64.06 ±1.42^{aA}
GTA_{cp}	56.18 ±0.11^{aA}	16.34 ±1.06^{aA}	63.67 ±1.21^{aA}	56.19 ±0.69^{aA}	19.44 ±1.72^{aA}	63.28 ±0.87^{aA}

Small letter = indicative the significant difference ($P < 0.05$) between the oleogels (in the same column)
Capital letter = Indicative the significant difference ($P < 0.05$) of the same oleogel on the same parameter at different crystallization period (in the same row)
*No significant difference detected at $P<0.01$

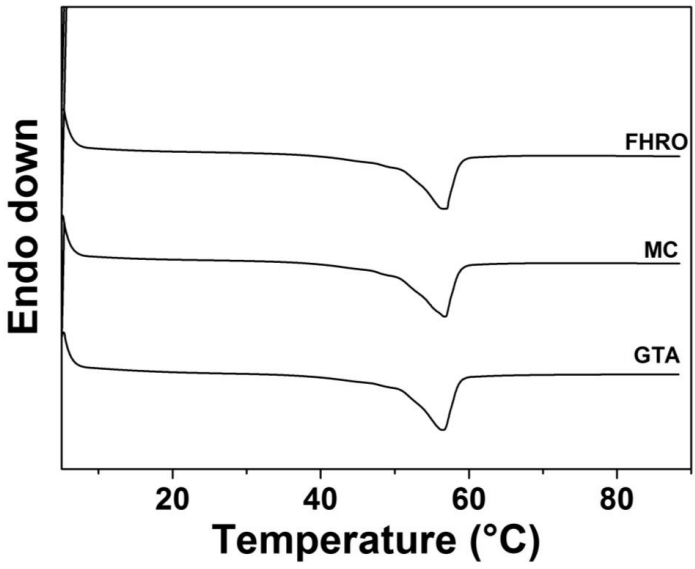

Figure 2.14 The melting thermogram of the Fully hydrogenated rapeseed oil (FHRO), Methylcellulose (MC)-based, and Gelatin (GTA)-based oleogels stabilized at 5°C for at least one week.

2.4.2.2.2 Diffraction properties of oleogels

Understanding the polymorphic type of fat-based products is critical as it determines the physical properties of the product (Douaire et al., 2014; Litwinenko et al., 2002; Marangoni et al., 2012). Therefore, the effect of MC and GTA on the molecular arrangement of FHRO was investigated using powder-XRD. XRD analysis will also elucidate whether the improvement in the rheological properties discussed above associates with the change in the polymorphic form or due to the presence of either MC or GTA as a co-structurant. There was no influence of MC and GTA on the polymorphic form of FHRO observed in the fat capsules, which was described in the earlier section (Section 2.4.1.3).

In general, there was no substantial difference in the molecular organization of polymer oleogels relative to FHRO oleogel (Figure 2.15). The only difference was observed in the shape of peak (intensity) but not in the peak position. Hence, the oleogels adopted similar packing, which resulted in a strong peak at 4.56Å, indicative of the formation of the β-polymorph and diffraction peaks at 4.2Å and 3.8Å which are indicative formation

of β' polymorph. These numerous diffraction peaks were also observed in a study comparing the polymorphic form of fully hydrogenated vegetable oils (Danthine & Deroanne, 2003; Deman, Deman, & Blackman, 1989). Comparable to the study done by Danthine and Deroanne, blending FHRO with different palm oil blends stabilized its β' polymorph, which has desired functionality in many fat-based products (Litwinenko et al., 2002; Narine & Marangoni, 1999), as in our study the β' form persisted even after one-week stabilization. Yet, complete transition from β' to β polymorph is possible during long stabilization but was not covered in this study. Overall, the diffraction pattern in oleogels is different with the pattern observed for the fat capsules (Section 2.4.1.3), though the stabilization time was similar. This difference is due to the lower mobility of molecules in fat capsules, which are predominantly composed of solids. The environmental factors such as viscosity and the presence of liquid medium facilitate the molecular mobility thus, can induce the rearrangement of molecular packing (Marangoni et al., 2012).

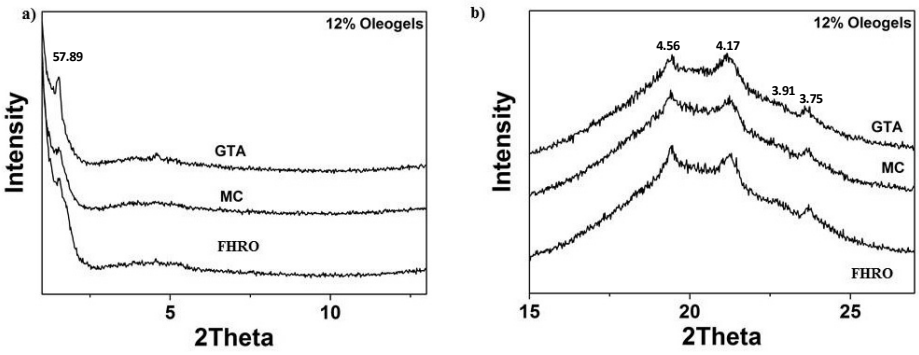

Figure 2.15 SAXD (a) and WAXD (b) of the Fully hydrogenated rapeseed oil (FHRO), Methylcellulose (MC)-based, and Gelatin (GTA)-based oleogels prepared by using the FHRO and fat capsules at 10 wt% in sunflower oil.

Based on the diffraction results, we can hypothesize that a composite oleogel network is formed, in which the crystals are organized independently without being influenced by the polymers. The same polymorphs were found in FHRO and in the polymer coated based oleogels. This indirectly proves the improvement in the rheological properties was not due to the difference in polymorph, but due to the presence of MC and GTA. Additionally, the diffraction patterns agree with the thermal behavior discussed previously (Section 2.4.2.2.1).

2.4.2.3 Morphology of oleogels

Using microscopic techniques, the structure of the oleogels prepared with the fat capsules was elucidated. The mechanical properties of fat crystal networks are influenced by the microstructure of the network formed (Litwinenko et al., 2002; Narine & Marangoni, 1999). The rheological analysis indicated that also the polymers contributed to the network formation. Therefore, it is necessary to visualize the location of polymer network and the morphology of the FHRO crystals and correlate these to the rheological properties.

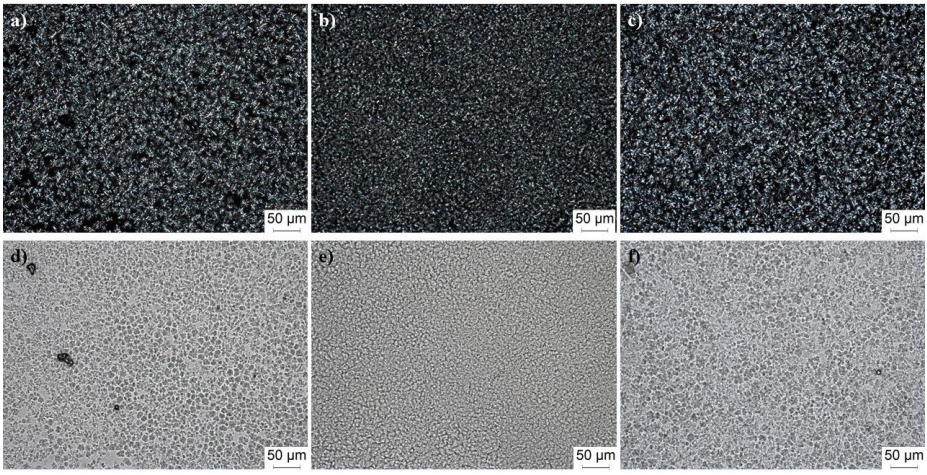

Figure 2.16. Morphology of the Fully hydrogenated rapeseed oil (FHRO) (a and d), Methylcellulose (MC)-based (b and e), and Gelatin (GTA)-based (c and f) oleogels visualized using polarized light microscopy (PLM) (top) and optical light microscopy (bottom).

The network structure was visualized by using PLM, SEM, and CLSM. Figure 2.16 illustrates substantial differences in the crystal network of the oleogels. The polymer based oleogels exhibited a condensed network of crystals, allowing the crystals to be spatially distributed through the oil which resulted in better rheological properties (Bin Sintang et al., 2017; Kouzounis et al., 2017; Tang & Marangoni, 2006a). This is in contrast with the FHRO oleogel which displayed large crystal aggregates that less effectively structured sunflower oil. There was also a clear difference in crystal size, which was smaller in the polymer based oleogels than in the FHRO oleogel. Cryo-SEM visualization showed similar microstructure of the oleogels prepared with the different

structurants (Figure 2.17). All the electron images exhibited a typical structure of fat crystals network, which appears as a continuous sheet (plate) of porous structure and in agreement with the electron images of shortening visualized by Heertje (1987). Heertje and co-workers explained that the continuous sheet of fat crystal network was aggregated of small plate crystals. However, the visualization of a single plate crystal was impossible as excessive ethanol extraction of oleogels dissolved the crystalline particles. This limitation was also discussed by Heertje (Heertje & Leunis, 1997; Heertje, Leunis, Vanzeyl, & Berends, 1987). The similarity in the structural pattern proves that the oleogels' network was dominated by a crystal network. However, the SEM image of GTA oleogel displayed a distinct structure connecting the crystal flocs (Figure 2.17c), which most likely was the GTA network. This is based on the appearance of the thin thread (filamentous-like) structure, which is typical for protein network (De Colli et al., 2012). Contrary to the other images (Figure 2.17a and 2.17b), whereby the continuous sheet appeared wide and platy. The MC oleogel did not reveal any polymer network formation, which could be due to the similarity in its appearance with the crystal networks.

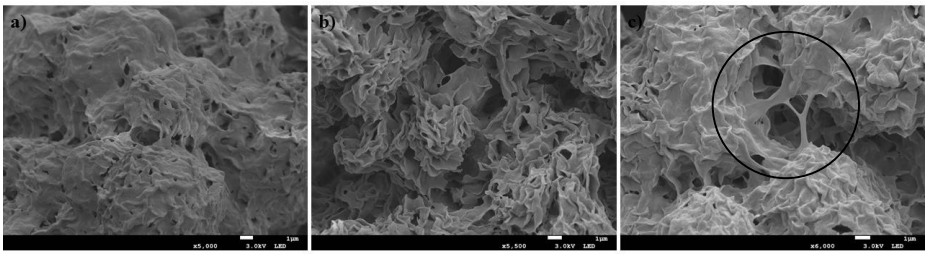

Figure 2.17 The morphology of the Fully hydrogenated rapeseed oil (FHRO) (a), Methylcellulose (MC)-based (b), and Gelatin (GTA)-based (c) oleogels visualized under scanning electron microscope (SEM). The scale bar is 1µm.

The polymer based oleogels were also visualized using CLSM. Only the polymers were stained, revealing their location in the samples. The preparation of oleogels for CLSM employed the same stained fat capsules, reported earlier (Figure 2.5 and 2.6). The dark background in Figure 2.18a and 2.18b represent the unstained area, which were fat crystals (FHRO) and sunflower oil. The green colour (fluorescent dyes) suggested the location of the polymers in the oleogels, which were labelled with the respective fluorescent dye. In the oleogels, strands of polymer were observed filling the spaces

between the crystal flocs (Figure 2.18). This image confirmed the image obtained in SEM of the GTA oleogel containing polymer strands connecting the crystal flocs. Although the presence of the polymers is confirmed, exactly how the polymers contribute to structuring remains unresolved. The polymers can function as co-structurant in a small bi-continuous network or as a strand connecting the crystals flocs of the FHRO, two options of which the latter is the most probable (Figure 2.16c). Though the polymer distribution is heterogeneous in the MC-based oleogel (2.17a), the heterogeneity is related to the size of fat capsules (Figure 2.3), since the fat capsules acted as a delivery agent for the polymers in this study. Therefore, it is proposed to optimize the fabrication of fat capsules to obtain a homogenous distribution, and as such improve the oil structuring properties and functionality.

The microstructure of the polymers visualized using different microscope is consistent with the thermal and diffraction results. It has been reported that the effect on crystallization will directly influence the microstructure/morphology of fat crystals (Martini, Puppo, Hartel, & Herrera, 2002). In this study, the presence of polymer does not influence the crystallization nor the morphology of the oleogels.

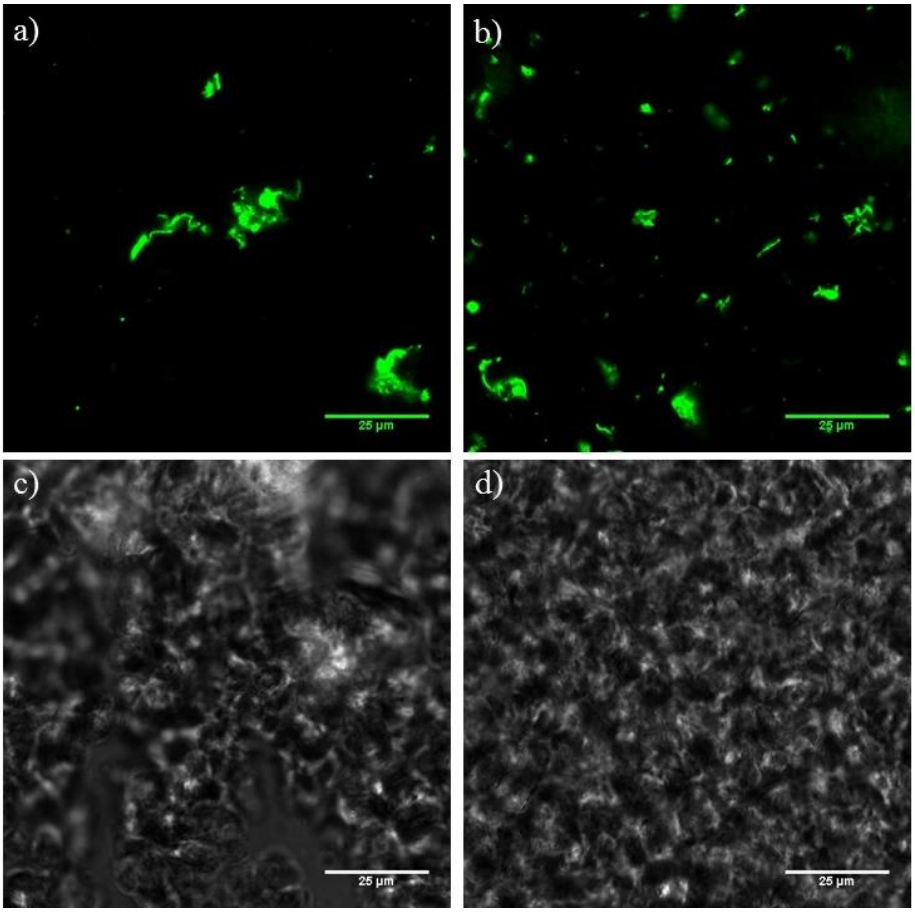

Figure 2.18. The confocal laser scanning microscopy (from one plane) of the Methylcellulose (MC)- based (a and c) and Gelatin (GTA)- based (b and d) oleogels. The a and b images represent the fluorescent, while c and d images represent the brightfield. The scale bar is 25 µm.

2.4.3 Combined effect of crystal network and polymer network in composite oleogel

Formation of a composite oleogel network consisting of fat crystal and polymer network is plausible. There are various scenarios to explain the mechanistic formation of the composite oleogel networks. One possibility is that the fat crystal network encapsulates or surrounds the polymers and clusters to form a mixed network. Another possibility is that there a bi-continuous, interpenetrating network is formed where both networks co-

exist and are unperturbed by the other (Chauhan et al., 2017a). Based on these two scenarios, it can be speculated that the oleogels are structured by the combination of crystal network as the main structurant and polymer as the co-structurant. . The formation of such a network could be inferred from the thermal behavior, diffraction analysis, and CLSM images. The presence of polymer networks or sheets that randomly entangle between the fat crystal network induces jamming (volume) effect. This jamming effect has been proven as a physical approach to influence the rheological properties of gels (Chauhan et al., 2017a; Chauhan et al., 2017b; Yoshikawa et al., 2015). In the respective studies, the addition of molecules that induce jamming did not influence the crystallization of the fat thus, indicating the improvement in the rheological properties was due to jamming effect. Similarly, this study showed that the polymers did not influence the crystallization of FHRO. Therefore, the jamming or volume effect is the plausible explanation in this study.

The successful integration of the fat crystal network with a polymer network provides different alternative to the current technique of emulsion-templated approach to incorporate hydrophilic polymers to edible oil (de Vries et al., 2015; de Vries, Wesseling, et al., 2017; Patel, Cludts, Bin Sintang, Lesaffer, et al., 2014; Romoscanu & Mezzenga, 2006; Tavernier, Patel, et al., 2017). In other words, this approach offers another alternative to incorporate hydrophilic polymers as a co-structurant for oleogelation field. Additionally, there is a growing interest to add colloidal particles/networks into fat crystals networks to induce jamming and form a space-filling network. Jamming influencing the interparticle interaction and consequently, the rheological properties. In this study, the concept is clearly demonstrated by the microscopic images, especially from confocal microscopy (Figure 2.18) where the polymers filled the spaces between fat crystals. Recently, Chauhan and co-workers reported the non-covalent interaction between silica particles was responsible to induce jamming in the fat crystals network of a composite network (Chauhan et al., 2017b). In their studies, the hydrogen bonding between the silica particles formed colloidal (silica) aggregates which back the fat crystals network. They also suggested that the added particles do not need to form a gel in oil to strengthen the fat crystal networks. Solely the addition of particles aggregates (at different spots) suffices to strengthen the network. However, the interaction between fat crystals and the silica aggregates remains vague. In the current understanding, they do not interact with each

other. Similarly, the hydrophilic polymers in the oleogels were present as a thin film at different spots within the fat crystal (FHRO) network. Thus, the same anology can be drawn between the study of Chauhan (2017a and b) and this study. Contrary to the silica approach by Chauhan (2017a and b), applying a hydrophilic polymer requires preliminary preparation, as most of hydrophilic polymers need to be hydrated to be functional (de Vries et al., 2015; A. R. Patel & Dewettinck, 2016). Therefore, the fabrication of fat capsules was performed to allow the polymers to form a network which was delivered to sunflower oil by the crystallized FHRO.

The fat capsules have proven to be capable of introducing the hydrophilic polymers into the liquid system. However, optimization is required to improve the preparation steps thus, improving properties of fat capsules. Further studies should focus on the distribution and optimization of the technique employed in this study. The spherical shape of fat capsules coated with hydrophilic polymer provides added advantage to the commonly produce spherical fat particles fabricated using microfluidizer (Kim & Vanapalli, 2013), in which the spherical particles are fabricated without the presence of a stabilizer. Thus, in combination with the reported fabrication technique (microfluidizer), micro(nano)particles with tailored properties (stabilizing layer) can be fabricated for a wide variety of different applications instead of solely as a structuring agent. Microparticles with tailored properties have a big potential in foods, cosmetics, and advanced materials applications (Kim & Vanapalli, 2013; Letourneau et al., 2005; Munuklu & Jansens, 2007).

2.5 Conclusion

Finding suitable structurants for edible liquid oil faces challenges because structurants are restricted by regulatory approval and properties can mismatch with oil. In this work, we adapted and modified the existing techniques of incorporating the hydrophilic polymers in the oil medium (Patel, Cludts, Bin Sintang, Lewille, et al., 2014; Romoscanu & Mezzenga, 2006). This study successfully developed the innovative approach of delivering hydrophilic polymers through the fabrication of fat capsules. The fat capsules were fabricated using a series of processing steps comprise of emulsification, creaming, and drying. In our approach, FHRO (internal fat phase) acted as a carrier for the hydrophilic polymers to be introduced in sunflower oil. The resultant fat capsules were spherical and had a protective polymer layer adsorbed at the

surface. This protective layer entangled in sunflower oil once the fat capsules were melted during oleogel preparation. The images obtained by a series of microscopy techniques proved the existence of the polymers in the capsules and oleogels. In the oleogel systems, the incorporation of the polymers enhanced its rheological properties compared to the systems structured using only FHRO due to jamming. Hence, the formation of a composite oleogel network comprising of fat crystal and polymer networks is proposed. The thermal behavior and diffraction peaks did not show any difference among the oleogels, regardless of the structurants used. These results in agreement with proposed of jamming of fat crystals network studied recently by Chauhan and co-workers (2017a and 2017b).

Although the structure of the oleogels still relies on TAGs crystals, the oleogels showed improved rheological properties in the presence of a co-structurant, as a polymer network. Therefore, this study provides manifestation of redesigning the structure of fat-based system to obtain desireable physical properties. Moreover, it is wise to say that this approach provides different alternative for influencing the physical properties of fat-based products, which is commonly done through chemical interesterification, addition of emulsifiers, or processing conditions (Acevedo & Marangoni, 2015; Sato et al., 2013; Sato & Ueno, 2011; Smith et al., 2011; Verstringe et al., 2014).

Ultimately, fabrication of polymer coated fat crystals adds another potential alternative structurant approach to structure liquid oil. Additionally, this study showed the success of the developed approach, fat capsules, as a carrier for hydrophilic polymers. The innovative approach of polymer coated fat crystals employed has great potential for further exploration from scientists and food industries. The potential is not limited to solely oil structuring but can be extended to delivery of functional materials, through incorporation in the internal fat phase. The diffraction and thermal studies on fat capsules manifested that the fabrication process and the presence of polymers did not influence the crystallization of the internal fat phase. These results implies the possibility of using fat capsules fabricated in this study as delivery vehicele of functional ingredients. It has been reported and discussed in literature that the lipid matrix has an influence on the efficiency of solid lipid particles as a delivery vehicle (O'Sullivan et al., 2016; Scalia et al., 2015).

As been outlined in the research strategy, this study is a proof-of-concept of the innovative approach. Thus, FHRO can be substituted to any crystallized species or even oil. Nonetheless, optimization in the fabrication process of fat capsules is necessary to obtain better fat microcapsules with broad applications, such as seeding crystals that simultaneously influence the physical behavior of products. Additionally, different approach of structuring or controlling the rheological properties of soft material could be achieved by dispersing fat capsules in sunflower oil, without having to melt the fat capsules. The intact fat capsules are dispersed in oil and interconnected between one another. With this approach, the presence of hydrophilic polymers at the interface helps to stabilize the fat capsules' network, forming capillary suspension. This type of structuring has been widely investigated by researchers in mechanical and chemical engineering (Bossler & Koos, 2016; Koos, 2014; Koos, Kannowade, & Willenbacher, 2014), and recently has been applied in food system (Hoffmann, Koos, & Willenbacher, 2014; Wollgarten, Yuce, Koos, & Willenbacher, 2016). In theory, the stabilization of fat capsules' network is based on capillary forces. However, the fabrication of capillary suspension requires intensive optimization in the preparation steps such as mixing temperature and speed. Additionally, it is also important to fabricate fat capsules at the right particle size, colloidal size (~1 μm).

2.6 Perspectives

The preparation of fat capsules coated with a hydrophilic polymer and their application as an oil structuring agent merits further investigation. In Chapter 5, we used synchrotron radiation X-ray technique to elucidate the effect of MC on the crystallization of FHRO. The effect was studied during the cooling and melting process to investigate the effect on molecular organization and polymorphic transition. These results provide additional information on the molecular packing of the FHRO in the presence of MC.

From a general perspective, the fabrication of fat capsules requires optimization. The optimization can focus on the concentration of polymer in the aqueous phase, the water-to-oil ratio of emulsion, and drying technique. Techniques to produce fat nano/micro particles such as micronization equipment, and microfluidizer to name one, could be employed to fabricate polymer coated fat crystals. It is also suggested to incorporate oil into the internal fat phase to reduce dependency towards solid fat.

Chapter 3 Monoglycerides and phytosterols binary mixtures as oil structuring agents

Relevent publications:

Bin Sintang, M.D., Danthine, S., Brown, A., Van de Walle, D., Patel, A.R., Tavernier, A., Rimaux, T., & Dewettinck, K. (2017). Phytosterol-induced viscoelasticity of oleogels prepared by using monoglycerides. **Food Research International**, 100 (832-840)

Bin Sintang, M.D., Rimaux, T., Van de Walle, D., Dewettinck, K., & Patel, A.R. (2017). Oil structuring properties of monoglycerides and phytosterols mixtures. **European Journal of Lipid Science and Technology**, 119(3) 1438

Bin Sintang, M.D., Danthine, S., Tzompa, D., Van de Walle, D., Patel, A.R., Rimaux, T., & Dewettinck, K. Enhancing solubility of phytosterols by complexation with monoglycerides. **Will be submitted to Journal of Agriculture and Food Chemistry**

3 Monoglycerides and phytosterols binary mixtures as oil structuring agents

3.1 Research strategy/hypothesis

In this chapter, the potential of combinations of monoglycerides and phytosterols to structure sunflower oil was researched. Phospholipids (polar lipid) and sterols are often found together in the lipid cell membrane (Figure 3.1). In the membrane, sterol molecules seem to have a condensing or chain ordering effect on the acyl chains of the phospholipids. Therefore, we hypothesized that synergistic interactions could arise by combining monoglycerides (polar lipid) and phytosterols as structurants in sunflower oil.

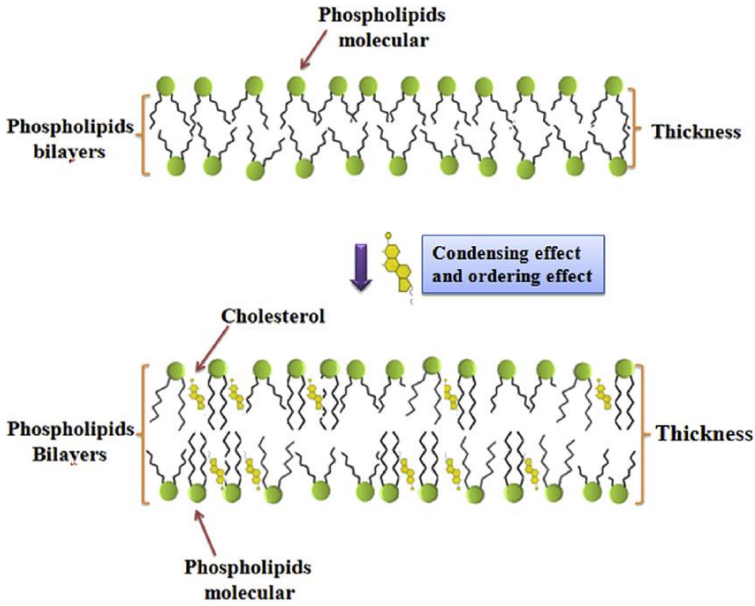

Figure 3.1. Schematic illustration of condensing effect/chain ordering of cholesterol on the acyl tails of phospholipids (Liu, Han, Zeng, Sun, & Aadil, 2016).

In this chapter, the mixing behavior of monoglycerides and phytosterols at different ratios was investigated. Complexes consisting of both molecules were prepared through ethanol precipitation, and based on the information from these complexes

such as ratio, oleogels were prepared. The final properties of these oleogels were investigated to understand their capability as oil structuring agents.

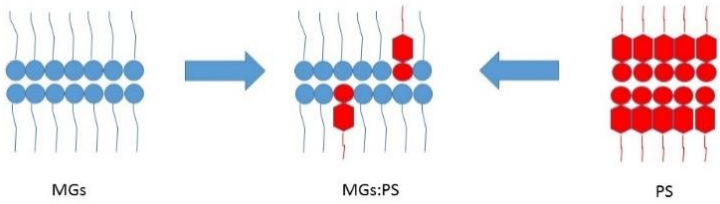

Figure 3.2. Schematic illustration of the hypothesis on the interaction between phytosterols and monoglycerides, condensing effect/chain ordering.

3.2 Introduction

Monoglycerides (MGs) are lipid molecules consisting of a single fatty acid esterified to the glycerol backbone. Different MGs vary in the length of their fatty acid carbon chain and in the number and position of the double bonds in the carbon chain. When dispersed in oil, MGs form an elastic gel upon cooling below the Kraft temperature (Chen & Terentjev, 2009; Ojijo et al., 2004; Verstringe, Moens, De Clercq, & Dewettinck, 2015). MGs can create four different phases when dissolved in a hydrophobic matrix (oil): isotropic, inverse lamellar, sub-α crystalline, and β-crystalline phases as shown in Figure 1.9 (Chen & Terentjev, 2009). Upon cooling, MGs first form an inverse lamellar phase in which the glycerol heads are densely packed in a hexagonal manner in planes in the middle of bilayers, also known as the α-phase. Further cooling leads to the transformation of the α-phase to the sub-α crystalline phase which is characterized by aliphatic chains packed in an orthorhombic configuration. The rheological properties of those two phases are similar. However, both phases are only metastable and tend to transform into a triclinic packing as a function of time, resulting in β-crystalline phase (Chen & Terentjev, 2009).

Monoglyceride oleogels in the β-crystalline phase exhibit large crystal aggregation, which compromises the oil binding capacity of the gel (Chen & Terentjev, 2009; Ojijo et al., 2004). Namely, the β-crystalline phase formation causes a separation of the D- and L-isomer of monoglycerides (Chen & Terentjev, 2009). Several studies have therefore focused on controlling the crystallization and polymorphic behavior of monoglycerides in oil to mitigate this undesired aggregation by, for example, applying

shear nanostructuring (Da Pieve et al., 2010) or by combining monoglycerides with ethylcellulose (Lopez-Martinez et al., 2015). Ethylcellulose can bind with monoglycerides through hydrogen bonding and as such modify its crystallization behavior. The addition of ethylcellulose also improves the rheological properties of oleogels (Lopez-Martinez et al., 2015).

Phytosterols (PSs) are naturally occurring cell wall stabilizing components in plants and are commercially used as a cholesterol-lowering agent. PSs crystallize into three different crystalline forms: anhydrate, hemihydrate and monohydrate, depending on the crystallization environment (Christiansen, Rantanen, von Bonsdorff, Karjalainen, & Yliruusi, 2002; von Bonsdorff-Nikander, Karjalainen, Rantanen, Christiansen, & Yliruusi, 2003). Much alike cholesterol (COH), PSs have a sterol based molecular structure consisting of a steroid skeleton with a hydroxyl group attached at the carbon number 3 (C-3) of the A-ring and aliphatic side chain at carbon number 17 (C-17) of the D-ring (Moreau, Kohout, & Singh, 2002). β-sitosterol and γ-oryzanol have shown to form unique structures in canola oil which are capable of trapping oil via capillary action between the self-assembled γ-oryzanol and β-sitosterol co-crystals (Bot & Agterof, 2006; Bot et al., 2008; Co & Marangoni, 2012).

Gamma-oryzanol and PSs are capable of co-crystallizing and they form hollow tubules with 10.9 nm diameter and 1.5 nm wall thickness, which explains why the combination is capable of immobilizing liquid oil (Bot et al., 2012). These tubules may co-assemble and create a continuous three-dimensional network at a macroscopic length-scale with the non-polar solvent internally immobilized (Bot & Agterof, 2006). PSs have also been combined with other compounds. For instance, Han and co-workers studied the structuring properties of sitosterol and lecithin mixtures. Their findings suggest that lecithin induced a change in the assembly of β-sitosterol in high linoleic sunflower oil which altered the physical properties of the corresponding oleogels (Han et al., 2014). Bot and Agterof examined different sterols and found that dihydrocholesterol, cholesterol, β-sitosterol, and stigmasterol produced firm oleogels (Bot & Agterof, 2006). They concluded that the presence of the hydroxyl group was critical for the formation of the gel. Additionally, the ring structure without double bonds accelerated the gel formation, whereas a double bond resulted in no gel formation (Bot & Agterof, 2006).

Sterols are naturally present in lipid membranes in which they affect the physical properties by condensing the alkyl tails of phospholipids (Cao, Tokutake, & Regen, 2003). Additionally, it has been reported that phospholipids or polar lipids form hydrogen bonds with sterols (Gater et al., 2013). Thus, the effect of sterols on the phospholipid membrane acts as a base to hypothesize that a combination of MGs and PSs may interact synergistically. These mixed-component oleogels might present better rheological properties and the aggregation of monoglyceride crystals (β-polymorph) during aging might be mitigated because of the interaction. In this chapter, we report on the mixing behavior and oleogelation properties of MGs and PSs in sunflower oil at different ratios. The influence of combining the MGs and PSs at different ratios and its relationship with the oil structuring properties were addressed and discussed. This combination is inspired by nature and as such it is an interesting food-grade oil structuring alternative to saturated fats. Moreover, this combination simultaneously deliver PSs in more solubilized form which melts at lower temperature than the corresponding pure PSs form.

3.3 Materials and methods

3.3.1 Materials

Refined sunflower oil and distilled monoglycerides from palm oil-Dimodan (DM) (C16:0; 44.95%, C18:1; 37.55%) were supplied by Vandermoortele Lipids N.V., Belgium. CardioAidTM (phytosterols including 50.4% β-sitosterol, 25% campesterol, and 15.3% stigmasterol) was received as gift sample from ADM (USA). The ethanol (CAS-nr.: 64-17-5) was purchased from Sigma Aldrich (Belgium).

3.3.2 Co-precipitation of monoglycerides and phytosterols from ethanol

Crystallization was achieved through co-precipitation, an approach widely used to study a molecular interaction of two different components (Tantipolphan, Rades, Strachan, Gordon, & Medlicott, 2006). First, solutions of DM and PSs were prepared separately by dissolving 1 g of solute (MGs or PSs) in 200 mL of ethanol. Then, the solute was dissolved by stirring the solutions for 45 minutes. Subsequently, mixtures at 10:0, 9:1, 8:2,7:3, 6:4, 5:5, 4:6, 7:3, 2:8, 1:9, and 0:10 ratios of DM to PSs were prepared by pipetting the appropriate amount of each solution to an empty beaker. The mixtures were stirred again for 10 minutes, and then transferred to an oven at 40°C to

evaporate the ethanol. The mixtures were allowed to precipitate for 48 hours. All the samples were then stored at 5°C.

3.3.3 Oleogels preparation

Oleogels of DM, PSs, and their combinations (10:0, 9:1, 8:2, 6:4, 6:0, and 0:10 (DM:PSs)) were prepared by mixing appropriately weighed amounts of materials with sunflower oil at 10 wt% of structurants. The mixtures were then heated to 100°C under continuous stirring for approximately 20–30min using a magnetic stirrer (Model EM3300T, Labotech, Inc., Germany) followed by cooling to room temperature for a period of 10min. The samples were stored at 5°C in a fridge until used further for characterization.

3.3.4 Thermal behavior

Details of the instrument and the sample preparation steps used for DSC analysis were described in Chapter 2.

The melting behavior of the complexes (after one-month stabilization) was analyzed by heating the pan from 5°C to 160°C at a heating rate of 5°C/min. Subsequently, the crystallization behavior of the melted complexes was investigated by cooling to 5°C with a cooling rate of 10°C/min.

For the oleogels, the pans were prepared by transferring at least 5mg of molten oleogels to a DSC pan which was then sealed hermetically. The pans were heated to 100°C and held for 10min to eliminate crystal history and a temperature scan was performed by cooling to 5°C, at a cooling rate of 10°C/min. The samples were then kept isothermal at 5°C for one-hour and three-hour to heating to 100°C, at a heating rate of 5°C/min. The same pans were kept at 5°C for one-week, and their melting profile was analyzed by heating from 5°C to 100°C at a heating rate of 5°C/min.

3.3.5 Small amplitude oscillatory stress

The instrument and software are described in Chapter 2.

3.3.5.1 Oscillatory temperature ramp and time sweep

The procedure is comprehensively explained in Chapter 2.

3.3.5.2 Amplitude stress sweep

The procedure is comprehensively explained in Chapter 2.

3.3.5.3 Frequency sweep

To evaluate the change on the viscoelasticity over time, frequency sweeps were performed on the stabilized oleogels after one-week stabilization at 5°C using a cross-hatched plate-plate geometry. The analysis was conducted by immediately transferring the stabilized-oleogels on the lower geometry, which had been set to 5°C. The gap was 1000μm and the frequency ranged from 0.1 to 1000 Hz. The oscillatory stress value was selected within the LVR region.

3.3.6 Optical light microscopy

To visualize the effect of combination on the microstructure, the oleogels were subjected to heating and cooling analyses. The preparation of slides is described in Chapter 2. The slides were heated to 100°C and kept isothermal for 10 minutes to eliminate crystal history. Then, the slides were then cooled to 5°C with a cooling rate of 10°C/min. Once the temperature reached 5°C, the slides were immediately transferred into the fridge to allow the oleogels to crystallize for one week. After one-week period, the microstructure of the oleogels were visualized under the microscope with the temperature stage was set to 10°C.

The instruments and procedure of visualization are comprehensively explained in Chapter 2.

3.3.7 Scanning electron microscopy

The instrument and procedure are comprehensively explained in Chapter 2.

3.3.8 Powder x-ray diffraction

The instrument and procedure are comprehensively explained in Chapter 2.

3.3.9 Statistical analysis

The procedure is comprehensively explained in Chapter 2.

3.4 Results and discussion

3.4.1 Mixing behavior of monoglycerides and phytosterols studied using complexes approaches

3.4.1.1 Melting and crystallization behavior of complexes

Evaluating the mixing behavior of the combined systems is vital to elucidate their thermodynamic stability and compatibility (miscibility). Moreover, the mixing behavior is an initial indicator of possible interactions. Binary complexes of DM and PSs at different ratios were prepared through co-precipitation from ethanol. Co-precipitation from a solvent has previously been employed to study the phase behavior of combinations of cholesterol and phospholipid, thus elucidating the effect of cholesterol on the phase transition of phospholipid (Tantipolphan et al., 2006). This approach demonstrates the effect clearly without any interference of solution, which usually causes the solvent (dilution) effect (Maleky et al., 2012).

The complexation between DM and PSs was investigated by evaluating the melting behavior of binary mixtures after one-month stabilization of the crystals precipitated from ethanol. As illustrated in Figure 3.3 and Table 3.1, we observed a change in melting temperature from ratio 9:1 to 1:9 (DM:PSs), which indicates the formation of complexes. Similar complexes were reported by Akashe and Miller (2001), and Perlman (2013), where the melting temperature of PSs dramatically changed when mixed with monoglycerides. The formation of DM-PSs complexes is attributed to their capacity to interact via hydrogen bonding (Gater et al., 2013; Perlman, 2013). These hydrogen bonds are formed between the hydroxyl group of PSs located at carbon number 3 with the carbonyl group and hydroxyl group of monoglycerides (Gater et al., 2013). Additionally, the hydroxyl group of PSs located at carbon-3 is also an important binding site for the formation of tubules in combination with γ-oryzanol (Bot, den Adel, et al., 2009).

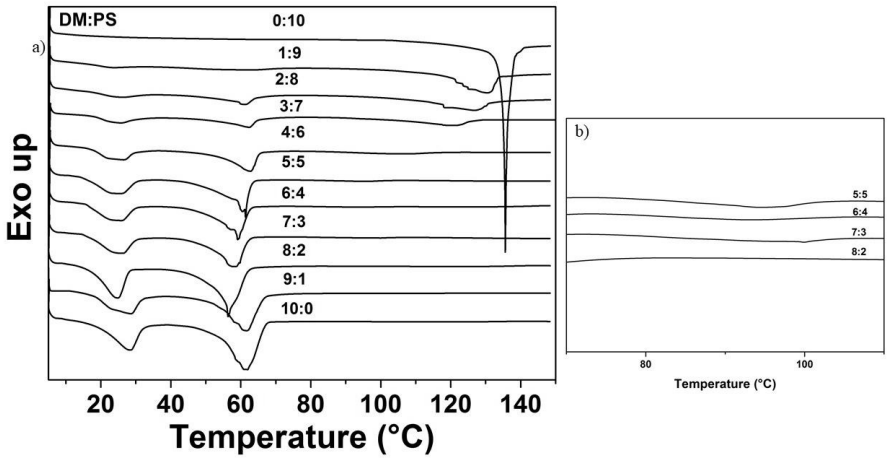

Figure 3.3. The melting thermogram of distilled monoglycerides (DM):Phytosterols (PSs) binary mixtures prepared through co-precipitation at different ratios (a) and the magnified thermogram of selected ratio (b). All the samples were stabilized at 5°C for at least a month.

Table 3.1 The offset temperature (T_{off}) and total enthalpy (H_{mp}) during melting of the distilled monoglycerides (DM):Phytosterols (PSs) complexes at different ratios stabilized for at least one-month (N=3 of independent sample).

Complexes (DM:PSs)	T_{off} (°C)	H_{mp} (J/g)
10:0	71.28±0.45$^{a\ (a)}$	155.20±7.53$^{f\ (d)}$
9:1	72.95±1.36$^{a\ (a)}$	131.20±5.12$^{e\ (c)}$
8:2	75.59±3.72$^{b\ (a)}$	130.30±5.01$^{e\ (c)}$
7:3	105.24±1.12$^{c\ (b)}$	105.70±6.17$^{d\ (b)}$
6:4	104.63±3.72$^{c\ (b)}$	98.36±6.21$^{cd\ (b)}$
5:5	103.12±1.61$^{c\ (b)}$	93.95±2.36$^{c\ (b)}$
4:6	117.72±2.34$^{d\ (c)}$	82.19±6.15$^{b\ (a)}$
3:7	129.54±1.21$^{e\ (d)}$	72.46±3.41$^{a\ (a)}$
2:8	134.30±2.16$^{f\ (e)}$	77.35±3.54$^{ab\ (a)}$
1:9	141.09±1.38$^{g\ (f)}$	78.31±3.45$^{ab\ (a)}$
0:10	141.08±1.39$^{g\ (f)}$	95.59±4.09$^{c\ (b)}$

Values indicated with different letters in the same column are significantly different (P < 0.05)
Values indicated with different letters, inside a bracket, in the same column are significantly different (P < 0.01)

In our study, the binary mixtures of DM at ratio 9:1 and 8:2 (DM:PSs) produced a single melting profile (Figure 3.3). This single melting profile indicates the presence of ideal mixing between DM and PSs, as both DM and PSs melted in the same temperature range. Conversely, the mixtures with higher PSs concentrations produced two separate melting profiles (7:3 to 1:9; DM:PSs). In those mixtures, the melting peak of

DM (61.44°C) was spotted even at 1:9 (DM:PSs) (Figure 3.3). The offset melting temperatures of the binary mixtures were significantly affected (Table 3.1). Moreover, the complexation of DM with PSs caused a reduction in the total melting enthalpy of complexes (Figure 3.4 and Table 3.1). These observations indicate that the formation of complexes significantly influences the melting temperature of PSs and affects the enthalpy of DM (Table 3.1). The effect of PSs on the melting temperature of DM was similar to the effect of cholesterol demonstrated on phospholipid (Mcmullen, Lewis, & Mcelhaney, 1994).

The ability of PSs to influence the melting temperature (Figure 3.3) and melting enthalpy (Table 3.1) in these mixtures is related to their ability to induce ordering in the hydrocarbon tails. The incorporation of low levels of sterols influences the hydrocarbon chain tilt required to provide a match (hydrophobic mismatch) between the hydrocarbon relative to the polar head group of DM, thus relieving the mismatch (Mannock, Lewis, & McElhaney, 2006). The cholesterol gradually pushes the adjacent hydrocarbon tails of the phospholipids apart in lipid membrane, which in turn allows the now slightly disordered hydrocarbon tails to assume a perpendicular orientation (Mannock, Benesch, Lewis, & McElhaney, 2015; Mannock et al., 2006), which loosely pack and are thus easy to melt. It has been discussed that the main transition in a pure bilayer (mono-component) of phospholipid is very sensitive to the hydrophobic interactions (de Meyer & Smit, 2009). However, we should note the difference between phospholipid and MGs in term of the polar cross-sectional area, which is larger in phospholipids. A large polar cross-sectional area causes a severe mismatch between the polar and lipophilic region. Thus, a slightly different effect might arise in our DM system. Furthermore, in a gel-state or below the sol-gel transition temperature, the sterols interfere with the packing of the hydrocarbon tails of respective polar lipid, for instance in phospholipid system (Smith, Wang, & Dea, 2012). Therefore, the crystallization behavior of the complexes was further characterized.

Investigating the crystallization behavior of mono-components and complexes prepared from ethanol precipitation further helps to understand the complexation of DM with PSs (Figure 3.4). The mixtures of 9:1 and 8:2 (DM:PSs) showed a suppression in the crystallization peak corresponding to the PSs (Figure 3.4). At binary mixtures from 7:3 to 1:9 DM:PSs, multiple crystallization peaks appeared. Additionally, the complexation also influenced the crystallization behavior of DM which indicates a two-

way interaction resulting from mixing DM and PSs (Figure 3.4). The onset crystallization temperatures of the 10:0, 9:1, and 8:2 DM:PSs were significantly different than the binary mixtures of 7:3 to1:9 DM:PSs (Table 3.2). Moreover, the total enthalpy of crystallization was significantly affected in the binary complexes (Table 3.2). Interestingly, the 9:1 and 8:2 complexes exhibited significantly lower enthalpy of crystallization than 10:0 DM:PSs, indicating the resultant effect of complexation.

Table 3.2 The onset temperature (T_{on}) and enthalpy (H_{cry}) during recrystallization of stabilized distilled monoglycerides (DM):Phytosterols (PSs) complexes at different ratios (N=3 of independent sample).

Complexes (DM:PSs)	T_{on} (°C)	H_{cry} (J/g)
10:0	45.46±0.60$^{a\ (a)}$	56.29±0.96$^{e\ (f)}$
9:1	41.83±0.83$^{a\ (a)}$	50.17±0.87$^{d\ (e)}$
8:2	40.15±0.42$^{a\ (a)}$	41.59±1.08$^{c\ (d)}$
7:3	59.31±6.04$^{b\ (b)}$	34.34±3.06$^{b\ (b)}$
6:4	64.51±1.67$^{b\ (c)}$	20.28±1.73$^{a\ (a)}$
5:5	66.17±10.05$^{b\ (d)}$	19.25±0.79$^{a\ (a)}$
4:6	85.15±2.87$^{c\ (e)}$	33.26±1.37$^{b\ (b)}$
3:7	107.39±1.15$^{d\ (e)}$	35.39±1.34$^{b\ (bc)}$
2:8	116.65±4.27$^{e\ (f)}$	40.92±1.30$^{c\ (cd)}$
1:9	124.45±5.43$^{f\ (fg)}$	56.13±5.61$^{e\ (f)}$
0:10	127.60±4.40$^{f\ (g)}$	67.02±4.24$^{f\ (g)}$

Values indicated with different letters in the same column are significantly different ($P < 0.05$)
Values indicated with different letters, inside a bracket, in the same column are significantly different ($P < 0.01$)

The same analogy with the phospholipid-sterol membrane system can be used to explain the crystallization behavior of the complexes (Bach & Wachtel, 2003; Mannock et al., 2015; Mannock et al., 2006). Below the main transition temperature of phospholipid, an interdigitated gel phase (L_β) is formed. However, in the presence of cholesterol, the formation of such phase is affected (Kamal & Raghunathan, 2012; Smith et al., 2012). This effect can be evaluated by assessing the liquid to gel transition temperature and thermal hysteresis (gap between melting and cooling) and the delay in crystallization (9:1 and 8:2 DM:PSs) (Halling & Slotte, 2004; Kamal & Raghunathan, 2012; Mannock et al., 2015; Mannock et al., 2006), which can be evaluated from Figure 3.3 and 3.5. The interference of the gel phase formation of DM was clearly seen from our results (Figure 3.3 and 3.4). Additionally, also the crystallization behavior of PSs was modified. PSs crystallized at a lower temperature than its corresponding pure component (Figure 3.4), which markedly decreased as the ratio of DM increased. The observable changes can be related to the complexation/interaction between DM and

PSs (Akashe & Miller, 2001; Perlman, 2013). Hence, the polymorphic forms of the resultant complexes were investigated to gain explanation for the change in the thermal behavior.

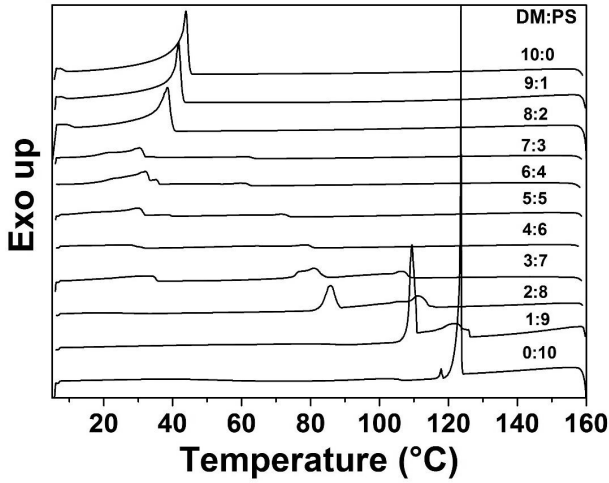

Figure 3.4. The crystallization thermogram obtained from the distilled monoglycerides (DM):Phytosterols (PSs) complexes prepared through co-precipitation.

3.4.1.2 Diffraction pattern of complexes

The investigation on the assembly and packing characteristics of DM, PSs and their combinations were performed on selected ratios of complexes. In the complexes, the WAXD patterns of DM mono-component revealed the presence of β polymorph, with a diffraction peak at 4.57Å which agrees with the reported pattern of β-polymorph (Da Pieve et al., 2011; Lopez-Martinez et al., 2014; Vereecken et al., 2009). In addition, the SAXD values indicated the double chain length of crystals formed through ethanol precipitation (Figure 3.5a). Contrary, PSs mono-component showed multiple diffraction peaks in SAXD and WAXD, corresponding to the crystallization into different crystal forms (Bach & Wachtel, 2003; Rossi, ten Hoorn, Melnikov, & Velikov, 2010; Vaikousi et al., 2007). The SAXD of PSs mono-component showed two separate peaks located between 37.52Å and 35.89Å (2.0 and 2.5° (2θ)). These diffraction peaks give the thickness of the pseudobilayer of repeating units of PSs (Bot & Flöter, 2011). In addition, the WAXD diffraction indicated the existence of monohydrate and hemihydrate crystals in PSs precipitated from ethanol. The existence of monohydrate

and hemihydrate forms can be detected with the appearance of peaks between 4.80Å and 4.73Å (18 and 19° (2θ)) (Bach & Wachtel, 2003; Bot & Flöter, 2011; Christiansen et al., 2002; Craven, 1976; Rossi et al., 2010).

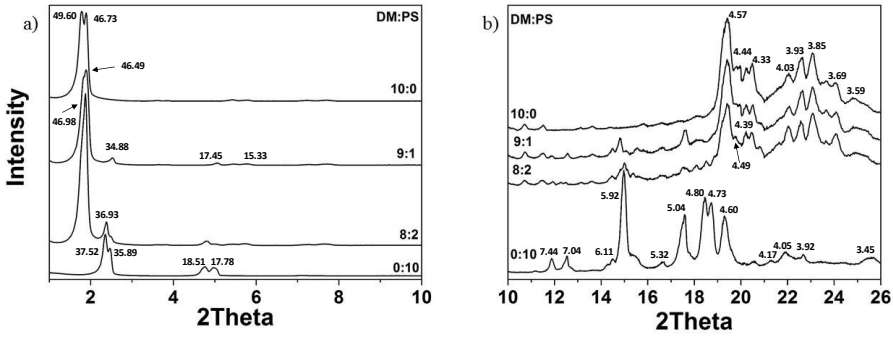

Figure 3.5 Small-angle x-ray diffraction (SAXD) (a) and wide-angle x-ray diffraction (WAXD) (b) pattern of distilled monoglycerides (DM):Phytosterols (PSs) complexes prepared through ethanol precipitation approach at different ratios.

The binary complexes of 9:1 and 8:2 DM:PSs clearly differed from their respective mono-components, indicating the formation of a new structure, characterized by different diffraction patterns in both SAXD and WAXD (Figure 3.5). The presence of PSs crystallites in the binary complexes is detected by the appearance of a peak at 34-37Å reflection (Bach & Wachtel, 2003). Thus, in the SAXD patterns of 9:1 and 8:2 complexes, the peak corresponding to PSs emerged as a single peak instead of two separate peaks observed in PSs mono-component (Figure 3.5a). The clearest indication for the formation of a new structure can be derived from the WAXD pattern. In the WAXD pattern of 9:1 and 8:2 mixtures, the peaks corresponding to the PSs were severely affected compared to the peaks of PSs mono-component (Figure 3.5b). The position of the peaks shifted, some peaks disappeared (2θ: 18-19 and 25-26), and new peaks emerged with respect to PSs crystal form (2θ: 2-3 and 20-21). The interference of hemihydrate and monohydrate peaks (4.73Å and 4.80Å) in the binary mixtures suggests the dominance of anhydrous form.

The formation of new peaks was also observed when PSs were crystallized using supercritical fluid, aerogels and in TAGs blends (Acevedo & Franchetti, 2016; Moreno-Calvo et al., 2014; Ribeiro et al., 2016; Rossi et al., 2010; Ubeyitogullari & Ciftci, 2016).

Generally, it can be concluded that the change in the diffraction was correlated to the formation of a new structure. Definition of a new crystal structure requires intensive crystallographic and spectroscopic analyses and requires further in-depth studies. Moreover, the SAXD peaks corresponding to DM merged into single peak in the binary complexes, while no substantial changes were observed in WAXD region. The change in the peak corresponding to DM in SAXD suggested the change in bilayer thickness, which could be attributed to the condensing effect/chain ordering by PSs. Therefore, the DM and PSs combinations display two-way effects, as the crystallization of both is affected.

The different polymorphic forms of PSs (anhydrous, hemihydrate, and monohydrate) is susceptible to the presence of water and impurities (Bot & Flöter, 2011; Christiansen et al., 2002; von Bonsdorff-Nikander et al., 2003). Impurities such as surfactants influence the recrystallization of PSs and other drugs because they adsorb to the hydrophilic faces of the growing crystals, which in turn influences the crystallization and polymorphic form, also known as crystal defect (Mackellar, Buckton, Newton, & Orr, 1994; Smith et al., 2011; von Bonsdorff-Nikander et al., 2003). This leads to increased secondary nucleation to provide new growing sites for other molecules to attach (Smith et al., 2011). As shown in the WAXD, the transition in the corresponding crystalline form of PSs could be explained to the role played by DM in controlling crystallinity (Figure 3.4 and 3.5).

The change in the thermal behavior of the PSs in the complexes can be linked to the change in the diffraction pattern. Particularly, in the observable effect of the diffraction peaks at 4.80 Å and 4.73 Å (18° and 19° (2θ)). Until now, there is no systematic study which defines the crystallographic structure of phospholipid-sterol or monoglyceride-sterol mixed crystals thus, limits our further interpretation (Akashe & Miller, 2001; Perlman, 2013).

3.4.1.3 Morphology of complexes

The complexes exhibited different thermal behavior and diffraction pattern than the corresponding mono-components, which is speculated due to crystal defect that influences crystal habit. Thus, the change in crystal habit (morphology) was studied using electron microscopy technique (von Bonsdorff-Nikander et al., 2003). Generally, in the complexes, DM showed plate-like structures of diverse widths (Figure 3.6a). The

PSs showed elongated plate-like structures with distinct in width and length dimensions (Figure 3.6d). These structures changed in the presence of DM, in which no observable PSs morphology was found in the binary complexes (Figure 3.6b and 3.6c). The binary complexes consistently showed only the characteristic of DM's plate-like crystals. Hence, we can hypothesize that the morphology of PSs changed because of complexation with DM. Several studies have been performed to investigate the role of stabilizers (emulsifiers) on the crystallization of PSs (Rossi et al., 2010; von Bonsdorff-Nikander et al., 2003) and these results agree with our findings.

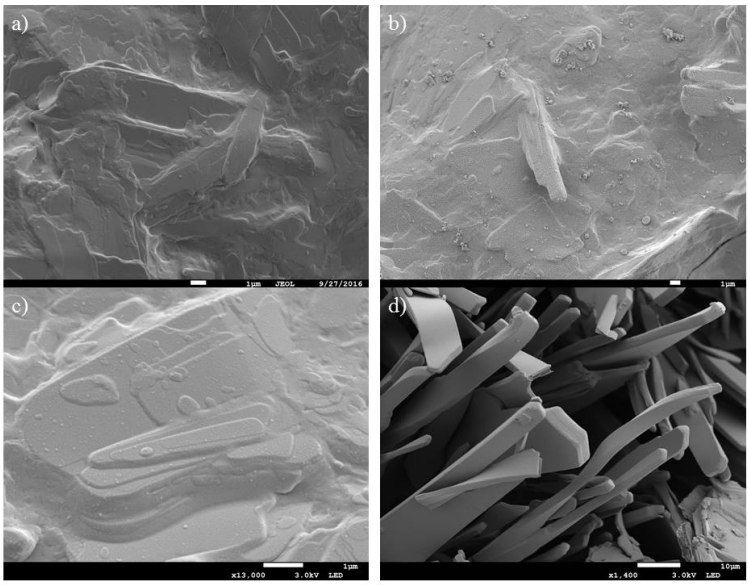

Figure 3.6 Electron microscopy images of distilled monoglycerides (DM):Phytosterols (PSs) complexes precipitated from ethanol at 10:0 (a), 9:1 (b), 8:2 (c), and 0:10 (d) DM:PSs.

3.4.2 Oil structuring properties of monoglycerides and phytosterols mixtures

3.4.2.1 Thermal behavior of oleogels

The crystallization behavior of oleogels prepared with DM, PSs, and their combinations was studied using differential scanning calorimetry (DSC). From the DSC thermogram in Figure 3.7 (Table 3.3), it can be derived that the oleogels display a concentration dependent crystallization, the onset crystallization significantly decreased with the change in ratio (Table 3.3). DM as only structurant in the oleogel crystallized at higher

temperature compared to the DM:PSs combinations. The peak crystallization temperatures and the enthalpy of crystallization showed a concentration dependent decrease with a decreasing proportion of DM (Table 3.3). DM in sunflower oil at low concentration (6:0 vs 10:0) produced slightly lower crystallization temperature (Figure 3.7), which is due to enhanced solubility in oil at lower concentrations (Ojijo et al., 2004). Yet, the crystallization temperature of 6:0 was higher than the 6:4 DM:PSs oleogel, despite the similar percentage of DM.

Table 3.3 The onset temperature (T_{on}), peak temperature (T_{cry}), and enthalpy (H_{cry}) during crystallization of the distilled monoglycerides (DM):Phytosterols (PSs) oleogels (N=3 of independent sample).

Oleogels (DM:PSs)	T_{on} (°C)	T_{cry} (°C)	H_{cry} (J/g)
10:0	$32.41 \pm 0.25^{c\ (c)}$	$29.83 \pm 1.12^{c\ (c)}$	$4.57 \pm 0.10^{d\ (d)}$
9:1	$31.89 \pm 0.54^{c\ (bc)}$	$30.25 \pm 0.59^{c\ (c)}$	$3.72 \pm 0.17^{c\ (c)}$
8:2	$30.53 \pm 0.62^{b\ (bc)}$	$26.78 \pm 1.39^{b\ (b)}$	$3.10 \pm 0.23^{b\ (b)}$
6:4	$26.75 \pm 0.14^{a\ (a)}$	$23.34 \pm 0.77^{a\ (a)}$	$1.98 \pm 0.20^{a\ (a)}$
6:0	$30.32 \pm 1.31^{b\ (b)}$	$26.78 \pm 0.79^{a\ (a)}$	$2.06 \pm 0.09^{a\ (a)}$

Values indicated with different letters in the same column are significantly different ($P < 0.05$)
Values indicated with different letters, inside a bracket, in the same column are significantly different ($P < 0.01$)

Chen and co-workers studied the crystallization behavior of saturated monoglycerides and assigned the first crystallization peak to the formation of inverse lamellar phase (α-crystalline), in which the polar heads face towards each other to avoid contact with non-polar solvent. This arrangement shows no direct contribution of the aliphatic chain, as it involves only the polar head groups (Chen & Terentjev, 2009). Further cooling to 5°C resulted in a transition from Lα to sub-α, which now involves the packing of aliphatic tails of saturated monoglycerides (Chen & Terentjev, 2009; Lopez-Martinez et al., 2014; Vereecken et al., 2009).

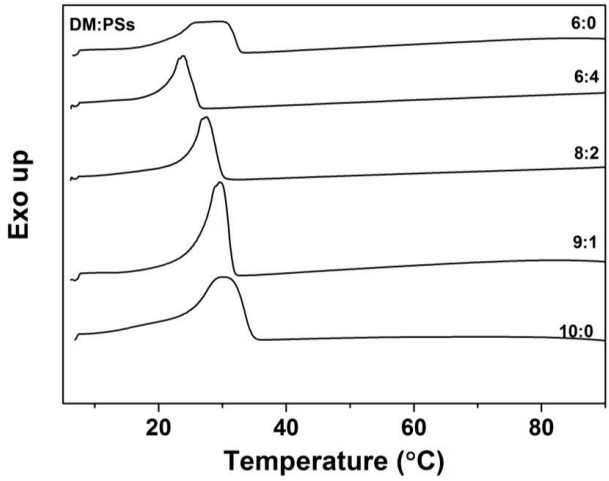

Figure 3.7 DSC thermogram of the distilled monoglycerides (DM):Phytosterols (PSs)oleogels crystallized to 5°C from melt at a cooling rate of 10°C/min.

During the crystallization of the oleogels, only one broad peak appeared, which is related to the formation of inverse lamellar, α-crystals (Figure 3.7). The DM:PSs oleogels at the studied ratios exhibited no sub-α peak formation, as was reported in literature (Chen & Terentjev, 2009; Lopez-Martinez et al., 2014; Vereecken et al., 2009; Verstringe et al., 2015). Lopez-Martinez, et al. (2014) used monoglyceride containing predominantly stearic acid (C18) for which the sub-α transition (crystallization of aliphatic chains) occured approximately at 10.38°C (Lopez-Martinez et al., 2014) in oil. However, the monoglycerides used in the current study is a mixture of palmitic acid and oleic acid, which requires higher degree of supercooling or longer isothermal crystallization at 5°C to induce the sub-α transition.

As more DM was substituted with PSs at fixed total concentration, the crystallization temperature was significantly depressed to an even lower temperature (Figure 3.7 and Table 3.3). The crystallization of lower temperature is correlated to the interference with the inverse lamellar formation due to intermolecular hydrogen binding (complexation) between PSs and DM (Chen & Terentjev, 2009; Gater et al., 2013; Lupi, Greco, et al., 2016). This effect was also observed in the mixing behavior described in the section 3.4.1. Interestingly, the 6:0 ratio showed a higher crystallization temperature than the 6:4 DM:PSs oleogel (Figure 3.7). This observation

clearly points out the putative interaction between the PSs and DM, especially in the crystallization process. During crystallization, the PSs may interact with the DM molecules and they solubilize together in the lamellar structures of DM (Crilly & Earnshaw, 1983; Michalak, Muzzio, Milianta, Giacomini, & Lee, 2013). As a result, PSs interfere with the arrangement of inverse lamellar structure of DM. Gater and co-workers studied the hydrogen bonding between cholesterol and monoglycerides in the lipid membrane thus, supporting the interaction between DM and PSs (Gater et al., 2013). Meanwhile, Larsson reported the change in the phase behavior of MGs in the presence of cholesterol as the result of interaction (Larsson, Gabrielsson, & Lundberg, 1978).

The effect of PSs on the polymorphic transition of crystals was studied using a thermodynamic approach, by measuring the evolution of melting temperatures as function of crystallization periods. Foubert and co-workers introduced the stop and return procedure using DSC to examine the polymorphic transition of fat crystals, without the need of X-ray diffraction (Foubert, Dewettinck, Janssen, & Vanrolleghem, 2006; Foubert et al., 2008). Moreover, Verstringe and co-workers applied isothermal crystallization to study the effect of monopalmitin on the crystallization of palm oil (Verstringe, Danthine, Blecker, Depypere, & Dewettinck, 2013). Using an approach inspired by both studies, the effect of PSs on the crystal structure of DM was investigated by evaluating the evolution in the melting temperature.

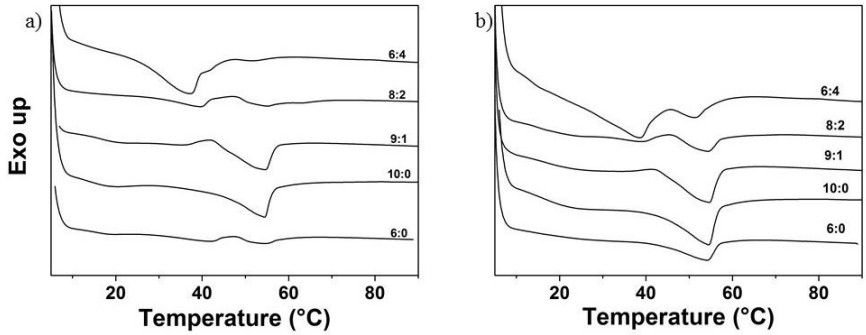

Figure 3.8 The graphs show melting thermogram of distilled monoglycerides (DM):Phytosterols (PSs) oleogels subjected to isothermal crystallization at 5°C for one-hour (a) and three-hour (b) period.

Figure 3.8 shows the melting profile of oleogels after one-hour and three-hour of isothermal holding times at 5°C, and the values are summarized in Table 3.4. After one hour, the 10:0 DM mono-component oleogel melted at a higher temperature followed by 9:1, 8:2, 6:0, and 6:4 (Figure 3.8a and Table 3.4). All the oleogels exhibited an increase in the melting temperature after three-hour isothermal period, which was significant in 6:4 DM:PSs (Figure 3.8b). Though the increase in the melting temperature signifies the progression in crystallization, the 8:2 and 6:4 DM:PSs oleogels consistently displayed two separate melting peaks (Figure 3.8b). It was clear from the crystallization analysis previously discussed that the presence of PSs influenced the crystallization process of DM. Likewise, the effect of PSs persisted in the growth phase (isothermal crystallization) as revealed by the difference in the melting temperature of bi-component compared to the mono-component oleogels.

Two explanations can be put forward to explain these results: i) the bi-component oleogels continued to crystallize during the isothermal period, but at a slower rate than the corresponding mono-component (enthalpy of melting Table 3.4), and/or ii) the presence of PSs affected the polymorphic transition of DM, which transitioned to a more stable polymorph, giving rise to a different melting temperature. The 10:0 DM:PSs oleogel melted at temperature similar to its melting temperature of one-hour isothermal time (Figure 3.8a and Table 3.4). The 6:0 DM:PSs oleogel on the other hand showed two separate peaks after one-hour isothermal, which merged into one broad peak after three-hour isothermal holding time (Figure 3.8a and 3.8b) caused by the different concentration. However, the 6:0 DM:PSs oleogel exhibited faster transition and significantly higher melting temperature than the 6:4 DM:PSs oleogel.

Table 3.4 The peak melting temperatures (T_{mp}) and melting enthalpy (H_{mp}) of the distilled monoglycerides (DM):Phytosterols (PSs) oleogels at different crystallization periods (N=3 of independent sample).

Oleogels (DM:PSs)	One-hour		Three-hour		One-week	
	T_{mp} (°C)	H_{mp} (J/g)	T_{mp} (°C)	H_{mp} (J/g)	T_{mp} (°C)	H_{mp} (J/g)
10:0	54.23 ±0.33^{bA}	7.84 ±0.41^{eA}	54.23 ±0.39^{bA}	10.06 ±0.57^{eB}	55.67 ±0.37^{bB}	11.53 ±1.72^{eB}
9:1	54.76 ±0.26^{bA}	6.41 ±0.43^{dA}	54.69 ±0.10^{bA}	7.06 ±0.41^{dA}	55.92 ±0.10^{bB}	9.27 ±0.40^{dB}
8:2	54.48 ±0.67^{bA}	3.31 ±0.09^{cA}	53.26 ±0.82^{bA}	4.66 ±0.13^{cB}	53.63 ±1.82^{abA}	7.97 ±0.76^{cdC}
6:4	36.68 ±0.62^{aA}	2.74 ±0.08^{bA}	51.25 ±0.05^{aB}	3.54 ±0.22^{bB}	51.04 ±2.52^{aB}	7.47 ±0.51^{cC}
6:0	54.30 ±0.33^{bA}	2.79 ±0.15^{bA}	54.16 ±0.53^{bA}	4.47 ±0.36^{cB}	54.15 ±0.20^{abA}	5.32 ±0.10^{bC}
0:10	69.59 ±2.33^{cA}	1.03 ±0.06^{aA}	71.06 ±2.23^{cA}	1.08 ±0.13^{aA}	73.46 ±4.77^{cA}	1.98 ±0.03^{Ab}

Small letter = indicative the significant difference (P<0.05) between the oleogels (Column)
Capital letter = Indicative the significant difference (P<0.05) of the same oleogel on the same parameter at different crystallization period (Row)
*Table A2 in the appendix provide the significant difference (P<0.01)

A visible difference in the melting profile of the 6:0 and 6:4 DM:PSs combinations was observed (Figure 3.8a, 3.8b, and Table 3.4). The melting temperature of 6:0 DM:PSs was significantly higher than 6:4 DM:PSs, indicating the influence of PSs on the polymorphism of DM. This means that the transition is faster in oleogel without PSs. Based on this result, the PSs not only affected the crystallization temperature but also the transition to a stable state. This result demonstrates the effect of PSs and supports the proposed explanation earlier.

After one week at 5°C, the melting profile of the oleogels was again analyzed, and demonstrated in Figure 3.9 (Table 3.4). The 10:0, and 9:1 DM:PSs showed significant increase in the peak melting temperature between three-hour and one-week periods. The change can be related to the change in peak shape, influencing the position of peak temperature. Moreover, the 8:2, 6:4, and 6:0 DM:PSs showed no significant change between the melting temperature for three hours and one week. For the 6:4 oleogel, the melting temperature increased, forming a broad melting peak which was identical to the melting pattern of 6:0 DM:PSs. Overall, the bi-combination oleogels melted at a temperature lower than 10:0 (DM:PSs) mono-component oleogel, though ample time and higher supercooling had been given to the oleogels.

Additionally, the melting enthalpy after one week was found to increase significantly than after one- and three-hour isothermal periods. This is particularly clear when comparing the melting enthalpy between three hours and one week of bi-component oleogels. The change in the melting enthalpy can thus be correlated to the contribution from the melting enthalpy of PSs, which separately crystallized. Moreover, the contribution from PSs in the bi-component oleogels is clearly seen as the melting enthalpy of 6:4 and 6:0 DM:PSs is significantly different at one-week isothermal storage (Table 3.4). However, the melting profiles (Figure 3.9) of the tested bi-component oleogels did not produce any visible peak which could be related to PSs.

For 0:10 DM:PSs oleogel, the melting profile produced a very small peak, which was difficult to discern when combined with the other peaks. The crystallization and melting profiles of the 0:10 DM:PSs are given in (Figure A2). Generally, the peak melting temperatures at different isothermal periods of PSs mono-component do not change significantly.

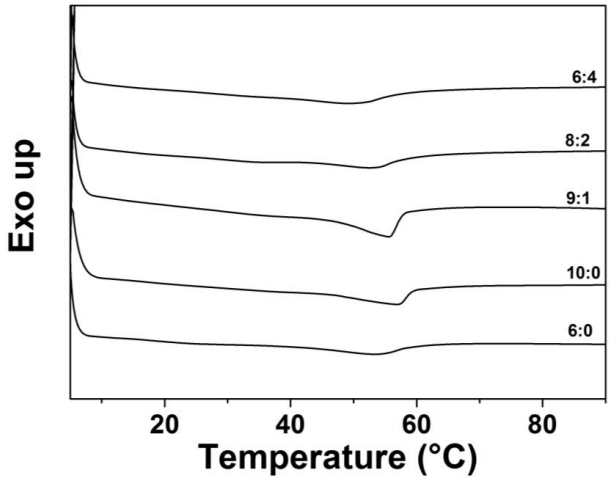

Figure 3.9 The melting profiles of stabilized distilled monoglycerides (DM):Phytosterols (PSs) oleogels at 5°C for one week.

A similar effect was observed in the thermal behavior of stearyl alcohol (SO) and stearic acid (SA) combinations (Blach et al., 2016). For certain ratios, the crystallization and melting peaks were shifted to a lower temperature than the corresponding SO and SA mono-component. The researchers correlated the change to the formation of a mixed crystal system. Likewise, the change in the crystallization and melting profiles of DM and PSs combinations may be attributed to the complexation between DM and PSs that led to the formation of mixed crystals. The authors hypothesized that the resultant intermolecular hydrogen bonding interferes with the crystallization and melting behavior, as also observed in bi-component oleogel of oleic acid and sodium oleate (Blach et al., 2016; Nikiforidis et al., 2015).

Our thermal investigation results are in agreement with the results found in the literature (Kamal & Raghunathan, 2012). Kamal and Raghunathan studied the phospholipid-phytosterol membranes system and showed that the presence of sterols lowered the main transition temperature (Kamal & Raghunathan, 2012), indicated with the depression in crystallization temperature.

3.4.2.2 Rheological behavior of oleogels

The oil structuring properties of DM, PSs and their combinations were studied by means of oscillatory temperature ramp, time sweep, amplitude sweep, and frequency sweep. Oscillatory temperature ramp and time sweep allow to evaluate the crystallization and microstructural development of crystalline-based system, as was studied by De Graef and co-workers (De Graef, Goderis, Van Puyvelde, Foubert, & Dewettinck, 2008). Plant sterols are known to act as regulators of the physical properties of the phospholipid membrane (Cao et al., 2003). In addition, the previous sections have shown the effect of PSs on the crystallization of DM, which might also affect the rheological behavior of the corresponding oleogels.

At a cooling rate of 10°C/min, the oleogels exhibited diverse gelation temperatures, which are identified at a δ-value of below 45° (Lupi, Greco, et al., 2016) (Figure 3.10 and Table 3.5). The temperature of gelation was ratio dependent, alike with the crystallization temperature measured using DSC. High ratio of DM showed significantly faster gelation than the bi-component oleogels, though the total concentration of structurants remained the same.

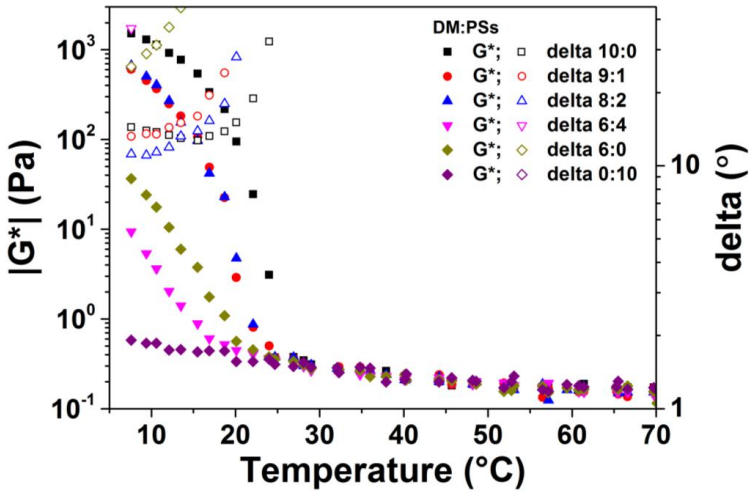

Figure 3.10 The evolution in the complex modulus and phase angle as function of temperature for oleogels prepared from distilled monoglycerides (DM), Phytosterols (PSs), and their combinations. The analysis was conducted with cooling rate of 10°C/min.

As expected, the 6:0 oleogel gelled at temperature lower than the 10:0 DM:PSs (Figure 3.10). This might be due to the difference in concentration (Ojijo et al., 2004). However, the gelation temperature of 6:0 was higher than the 6:4 DM:PSs, as was observed in the crystallization study. Therefore, it can be said that the complexation and intermolecular hydrogen bonding influences the self-assembly of DM, leading to slow gelation, manifested in the crystallization and gelation of 6:4 DM:PSs oleogel. Moreover, all the oleogels experienced an increase in the |G*| with decreasing temperature (at the end of temperature-ramp).

Table 3.5 The gelling temperature (T_{gel}) of the distilled monoglycerides (DM):Phytosterols (PSs) oleogels during temperature ramp (N=3 of same oleogel)

Oleogels (DM:PSs)	T_{gel} (°C)
10:0	25.33±0.84^{c}
9:1	20.33±0.50^{b}
8:2	21.23±0.38^{b}
6:4	13.57±2.04^{a}
6:0	14.63±0.78^{a}

Values indicated with the same letter are not significantly different at ($P < 0.01$)

The microstructural development of the oleogels were monitored by means of oscillatory time sweeps. At 5°C, similar pattern in the microstructural development between 10:0 and 6:0 DM:PSs was observed, with the former had higher |G*| due to the different concentration (Ojijo et al., 2004). However, there was a considerable variation in the |G*| of bi-combination oleogels (Figure 3.11 and Table 3.6). The bi-component oleogels showed a slow increase in the |G*|. This slow development in the |G*| agrees with the effect of PSs on the crystallization and transition which were discussed in the previous section. Overall, the 9:1, 8:2, and 6:4 DM:PSs oleogels produced similar pattern in the evolution of |G*|.

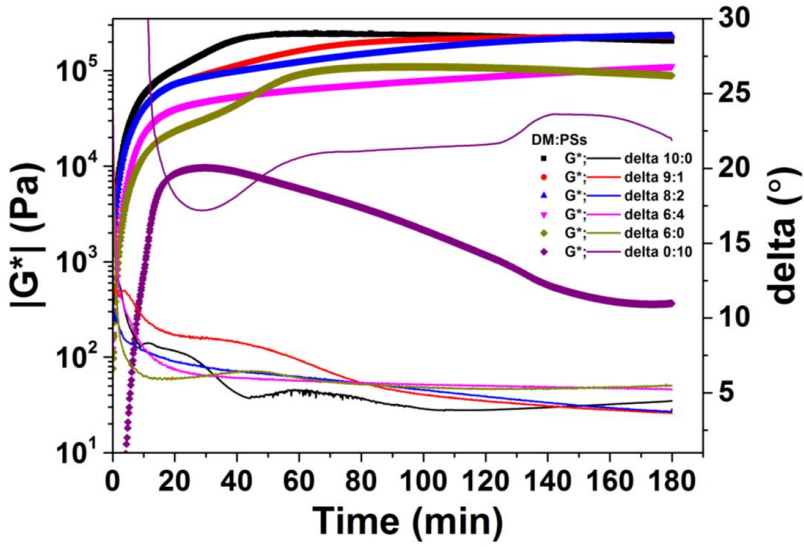

Figure 3.11 The graph shows the evolution in the complex modulus and phase angle of the distilled monoglycerides (DM):Phytosterols (PSs) oleogels at 5°C. The analysis was performed for three hours immediately after the temperature sweep.

The microstructural development of 0:10 DM:PSs was investigated to have an insight into the gelation process of PSs. PSs provides a structural building block to sunflower oil, which is evident in the high |G*| with low phase angle (Figure 3.11). However, the 0:10 DM:PSs experienced a drop in the |G*| as a function of time sweep. Co and Marangoni reported that solely PSs failed to structure edible liquid oil, as the crystals are prone to contraction and settle to the bottom (Co & Marangoni, 2012).

Table 3.6 The phase angle (δ) and complex modulus |G*| values of the distilled monoglycerides (DM):Phytosterols (PSs) oleogels at the end of three-hour time-sweep (N=3 of same oleogel).

Oleogels (DM:PSs)	Phase anlge/Delta (°)	Complex modulus |G*| (kPa)
10:0	4.33±0.12$^{b\ (a)}$	202.93±8.00$^{c\ (c)}$
9:1	3.64±0.14$^{a\ (a)}$	229.50±6.35$^{d\ (d)}$
8:2	3.92±0.45$^{ab\ (a)}$	230.93±5.44$^{d\ (d)}$
6:4	5.24±0.53$^{c\ (b)}$	111.21±14.20$^{b\ (b)}$
6:0	5.51±0.08$^{c\ (b)}$	89.13±1.19$^{a\ (a)}$

Values indicated with different letters in the same column are significantly different (P < 0.05)
Values indicated with different letters, inside a bracket, in the same column are significantly different (P < 0.01)

The influence of PSs on the formation of structural building block of DM is clearly manifested in the delay in microstructural development, indicated with slow increase in |G*| of bi-component oleogels as shown in Figure 3.11 (Table 3.6). A similar pattern of microstructural development was obtained by Kouzounis and co-workers (Kouzounis et al., 2017). The presence of PSs seems to influence the viscoelasticity by inducing chain ordering on the acyl tail of DM (Bach & Wachtel, 2003; Crilly & Earnshaw, 1983; Larsson et al., 1978; Michalak et al., 2013). The alignment of hydrophobic part of PSs molecules parallel to the acyl tail of DM influences the packing of DM (Smith et al., 2012) thus, affecting the molecular packing (crystallization/gelation). This effect changes the viscoelastic properties of the bi-component oleogels by affecting the microstructural development. The trend of microstructural development manifested by the bi-component oleogels agrees with the result of melting behavior described in the previous section, which also indicated a slow increase in melting temperature.

Over time, the chain ordering by PSs on the alkyl tail of DM resulted in an oleogel with better viscoelasticity as demonstrated by the higher |G*| at the end of three-hour time sweep (Figure 3.11 and Table 3.6). The 9:1 and 8:2 DM:PSs oleogels exhibited higher |G*| and lower δ than the 10:0 DM:PSs oleogel, indicating better oil structuring properties. Interestingly, the |G*| of 6:4 DM:PSs oleogel were higher than 6:0 DM:PSs, though the former exhibited low melting profile (two separate peaks) than the latter (Figure 3.8b). These results clearly imply that complexation and chain ordering induced by PSs on the acyl tail of DM improves viscoelasticity of oleogels. Similar improvement was reported by (Crilly & Earnshaw, 1983; Larsson et al., 1978), in which cholesterol influenced the viscoelasticity of monoglyceride bilayer. Although PSs initially interfere the gelation of the bi-component oleogels, the effect seems temporary as the 9:1 and 8:2 DM:PSs oleogels finally yielded higher |G*| than DM mono-component (Table 3.6).

An amplitude stress sweep test was performed on the same samples at the end of the time sweep, and the results are depicted in Figure 3.12. All the oleogels produced similar trends of G' with the |G*|. The 9:1 and 8:2 DM:PSs oleogels exhibited higher G' with longer linear region than the other bi-components. Additionally, the 6:4 DM:PSs oleogel had a higher G' and longer linear region than 6:0 DM:PSs oleogel, suggesting the contribution of the interaction between the PSs and the DM to the viscoelastic properties (Figure 3.12).

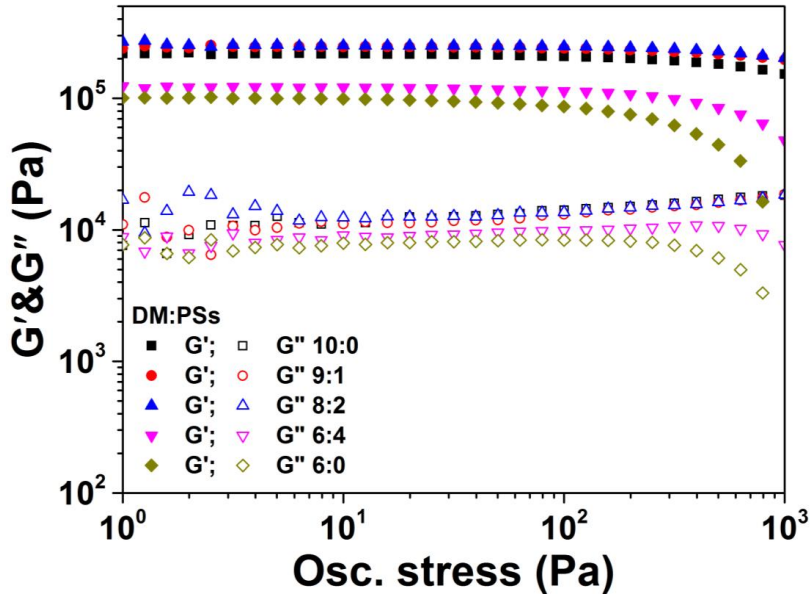

Figure 3.12 The amplitude stress sweep of the distilled monoglycerides (DM):Phytosterols (PSs) oleogels was conducted continuously after the three-hour time sweep at 5°C.

Further viscoelastic characterization was performed on the oleogels after one-week stabilization at 5°C by means of a frequency sweep (Figure 3.13). The highest G' was seen in the 9:1 and 8:2 (DM:PSs) oleogels compared to the other ratios (Figure 3.13), confirming the results of amplitude sweeps (Figure 3.12). The PSs mono-component oleogel showed phase separation after one week and was excluded from frequency sweep test. The overall increase in the G' of 9:1 and 8:2 DM:PSs oleogels over mono-component DM gel suggests a persistent effect of PSs.

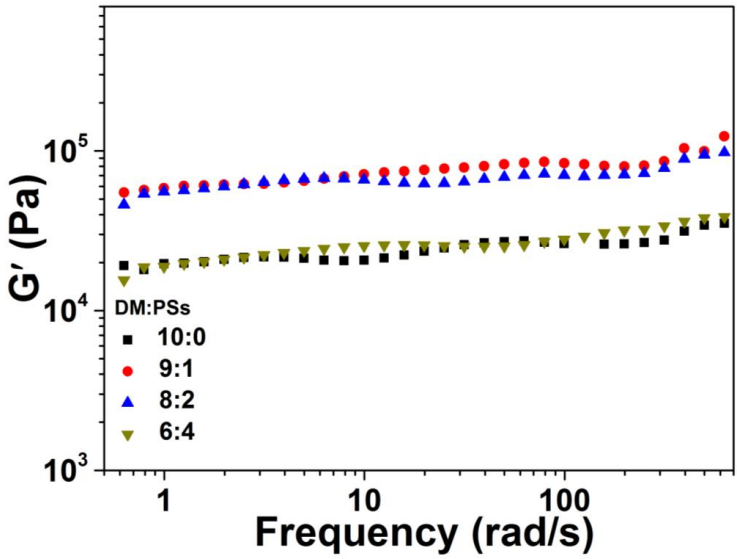

Figure 3.13 The frequency sweep of selected the distilled monoglycerides (DM):Phytosterols (PSs) oleogels after one-week stabilization at 5°C.

Ultimately, this study found that PSs temporarily interfered with packing of DM's acyl tail, resulting in low |G*|. Subsequently, over time, DM packed analogous to its mono-component, though slower than mono-component, indicated with higher |G*|, and substantially similar melting behavior of bi-component oleogels (Figure 3.11, 3.12, and 3.13). Hence, the complexation (condensing effect) was found to influence the packing/self-assembly and viscoelasticity. Up to date, the effect of sterols on phospholipid/monoglyceride bilayer is still a subject of debate. Some researchers stated that sterols will influence the local packing of bilayer at gel state and compromising the physical properties (Smith et al., 2012). However, that study was limited to a liposome system (vesicle) with short stabilization time, and only limited studies are investigating the effect in lamellae (crystalline) system. Thus, the rheological results presented in this section can be considered as pioneer for crystalline system. The results of amplitude stress sweep and frequency sweep found in this study agree with the reported results of monoglyceride and phytosterols bi-component oleogels (Yang, Chen, & Yang, 2017).

In this system, it is speculated that the structural building blocks consist of crystals from both DM and PSs, forming mixed crystals system (Blach et al., 2016). The mixed

crystals system is responsible for better network formation, in which PSs help to improve spatial distribution of DM crystals. Therefore, the diffraction analysis was peformed to investigate tehexisence of mixed crystal system.

3.4.2.3 Diffraction properties of oleogels

The investigation on polymorphic forms of DM, PSs and their combinations were performed on selected ratios of oleogels and demonstrated in Figure 3.14. The analysis was then used to compare the change in the polymorphic forms in liquid oil medium and in the complexes. Similarly to the complexes, different diffraction patterns were observed in the analyzed oleogels. All the oleogels exhibited the β-polymorph, as can be derived from the diffraction peak at 4.59Å (Da Pieve et al., 2011). In addition, the bi-combination oleogels showed diffraction peaks from both components, demonstrative of the formation of a mixed crystal system. As observed in the complexes, PSs also crystallized separately in the oleogels as peaks were observed between 2.0 and 2.5° (2θ) (Bach & Wachtel, 2003). However, PSs crystallized into a different polymorph in the 8:2 bi-combination oleogel. The 8:2 (DM:PSs) exhibited a bilayer thickness smaller than 0:10 and 6:4 (DM:PSs), as the SAXD diffraction appeared at high angle (2.8° = 34.36Å) (the corresponding peak for PSs). Additionally, no peak appeared for the 8:2 combination at 5.9Å compared to 6:4 and 0:10 (DM:PSs). As previously discussed, the DM might have influenced the crystallization of PSs. Thus, the disappearance of the peak at 5.9Å indicates the effect of DM on the crystallization of PSs. Generally, the SAXD and WAXD diffraction did not substantially change for the peaks corresponding to DM, which agrees with the diffraction patterns of the complexes.

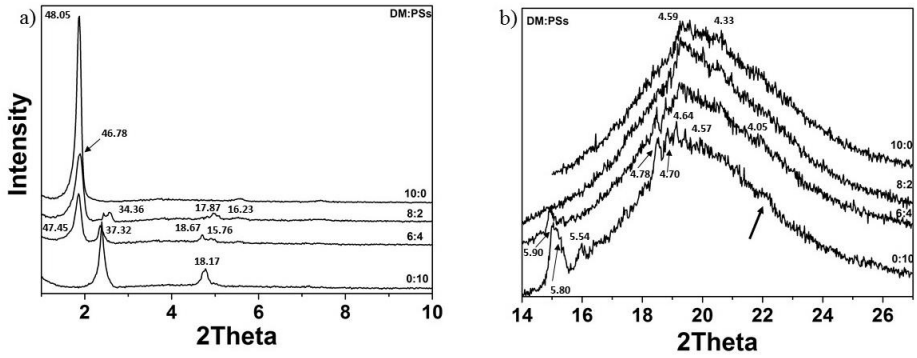

Figure 3.14 Small-angle x-ray diffraction (SAXD) (a) and wide-angle x-ray diffraction (WAXD) (b) pattern of selected distilled monoglycerides (DM):Phytosterols (PSs) oleogels after one-week stabilization at 5°C.

3.4.2.4 Morphology of oleogels

It has been demonstrated in the previous sections that DM and PSs interact by forming mixed crystals. Crystallization process influences the crystal morphology and the crystal network formation, which can be followed-up with microscopic techniques.

By means of optical microscopy, using both normal and polarized light, the stabilized oleogels were visualized. Based on our previous discussion, we hypothesized that when DM and PSs are combined, clustering of the DM crystals and aggregation of the PSs crystals are mitigated. The light microscopic images revealed the formation of network-like structures through the entanglement of crystals in all the oleogels. Dense crystals and crystal entanglement could retain the sunflower oil and limit the oil diffusion (Figure 3.15). In bi-components, the needle-like crystal network of containing both DM and PSs had a reduced size of clustered crystallites (spherulites) which is responsible for a promising rheological properties, higher |G*| (section 3.4.2.2). Large clustered crystals were observed in 10:0 DM:PSs oleogel (Figure 3.15a and 3.16a). The clustered crystals were formed through the aggregation of needle-like crystals, grown from a nucleation center and with several orders of branching, thus forming large crystal structures. These large crystals are associated with β-crystalline phase, which have been reported to compromise the oil binding ability of monoglyceride-based oleogels (Chen & Terentjev, 2009; Ojijo et al., 2004). The crystal morphology

observed in this DM-based oleogels agrees with the images reported in the literature (Da Pieve et al., 2010; Kesselman & Shimoni, 2007; Ojijo et al., 2004).

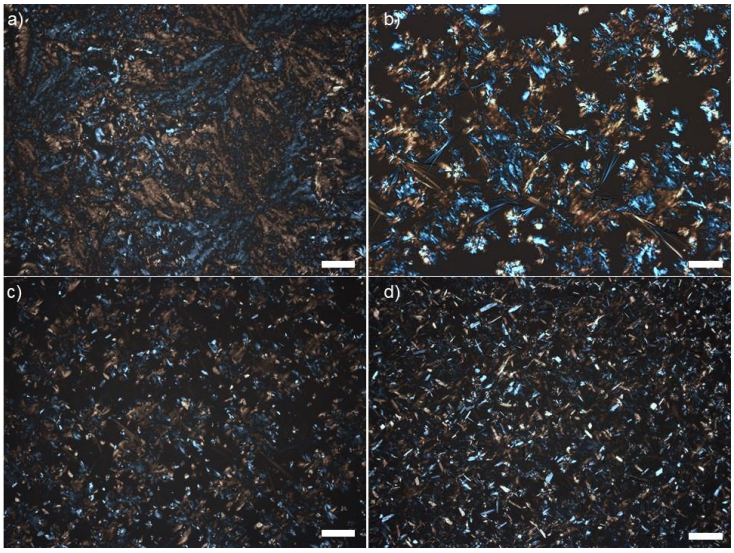

Figure 3.15 The morphology of crystals of 10:0 (a), 9:1 (b), 8:2 (c), and 6:4 (d) distilled monoglycerides (DM):Phytosterols (PSs) oleogels (scale bar = 100μm). The images of 6:0 DM:PSs were given in Figure A6.

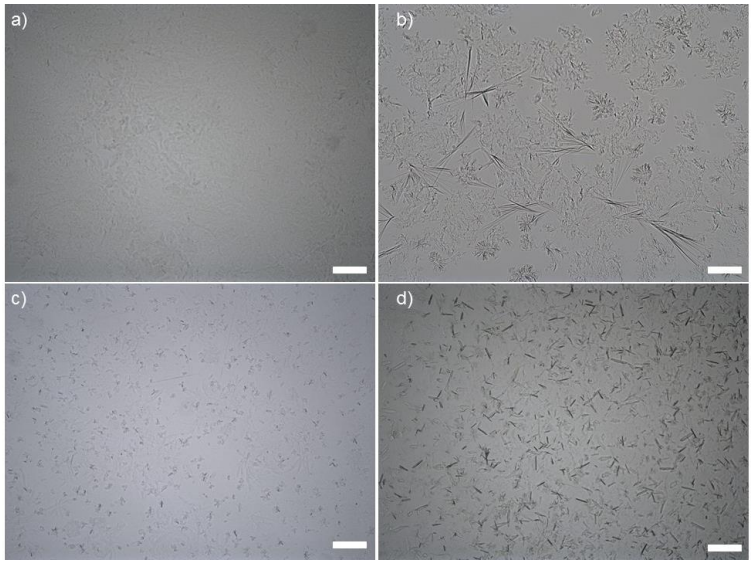

Figure 3.16 The optical light microscopy images of the 10:0 (a), 9:1 (b), 8:2 (c), and 6:4 (d) distilled monoglycerides (DM):Phytosterols (PSs) oleogels (scale bar = 100μm). The images of 6:0 DM:PSs were given in Figure A6.

PSs exhibited various structures including needle-like, spherulite and plate-like crystals and large aggregates (von Bonsdorff-Nikander et al., 2003) (Figure 3.17a and 3.17b). According to Blake and Marangoni (2015), these morphologies can be a different orientation of crystalline platelets (Blake & Marangoni, 2015). These morphologies agree with the contraction of needle crystals reported in the literature (Co & Marangoni, 2012), and observed during oscillatory time-sweep (Section 3.4.2.2).

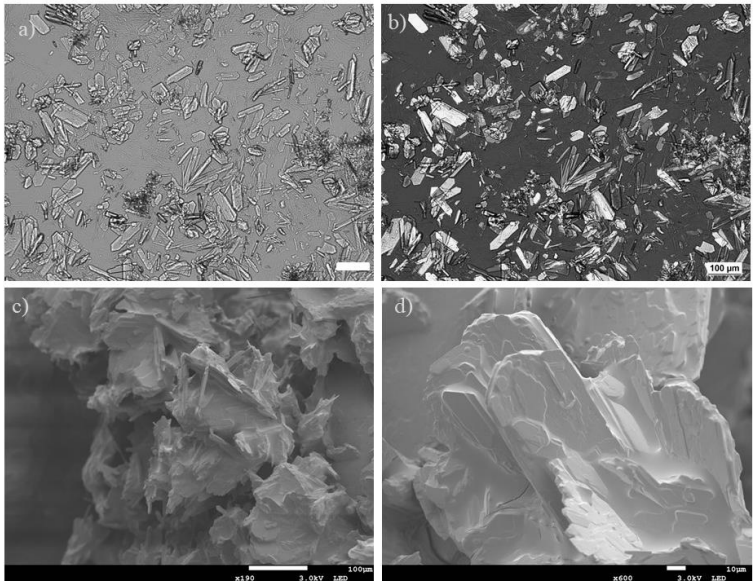

Figure 3.17 The crystal morphology of Phytosterols (PSs) oleogel (0:10) visualized using optical (a), polarized (b) (a and b have the same scale bar which is 100µm), and electron microscopy (c and d). Scale bar; c = 100µm and d = 10µm.

As seen from Figure 3.15b-d and 3.16b-d, the incorporation of PSs had two important effects: reduction of the large agglomeration crystals and disintegration of crystalline aggregates (spherulites) of DM crystals. The disintegrated crystals (which are mostly needle-shaped) resulted in better network formation due to a better spatial distribution (Kouzounis et al., 2017; Marangoni & Rousseau, 1996; Narine & Marangoni, 1999; Tang & Marangoni, 2006a). Additionally, the appearance of short needle-like crystals which resemble the morphology of PSs, supporting the hypothesis of mixed crystal system particularly clear in 6:4 (DM:PSs) (Figure 3.15d and 3.16d).

At low PSs ratio, 9:1 and 8:2 oleogels exhibited predominantly needle-like crystals structures with small clustered aggregates than the DM mono-component oleogel. As the PSs content increased, the volume of short needle-like crystals was increased alongside the DM crystals (Figure 3.15 and 3.16). Therefore, it can be said that there exists a threshold ratio above which the needle-like crystals of PSs aggregate together, forming large spherulitic structures. However, the large plate-like crystals of PSs were not observed in the bi-component oleogels, indicating that DM acted as a crystal habit

modifier (delaying the crystallization) as reported in the literature(Christiansen et al., 2002; Engel & Schubert, 2005). This is the fact that PSs at 5 %wt in sunflower oil form similar morphology as the 10 %wt (Figure A2).

With the electron microscope, an in-depth visualization of the internal structure was possible. The morphology of crystals in oleogels was found alike to the complexes (Figure 3.6 and 3.18). Figure 3.17c, 3.17d and 3.18 display the SEM images of the oleogel. The DM structure is composed of small-plate structures, characteristic of a lamellar structure (Acevedo, Peyronel, & Marangoni, 2011). These structures stack together to create hollow spaces that can entrap liquid sunflower oil (Verstringe et al., 2015). Acevedo and co-workers observed the same plate-like crystals, comprising of lamellar stacking (Acevedo & Marangoni, 2010; Acevedo et al., 2011; Ramel et al., 2016). The PSs mono-component oleogels exhibited identical morphologies observed under optical microscope. Yet, detailed structures were visualized using SEM, which showed clearly the dimension of plate-crystal of PSs (larger than~10μm) (Figure 3.17d).

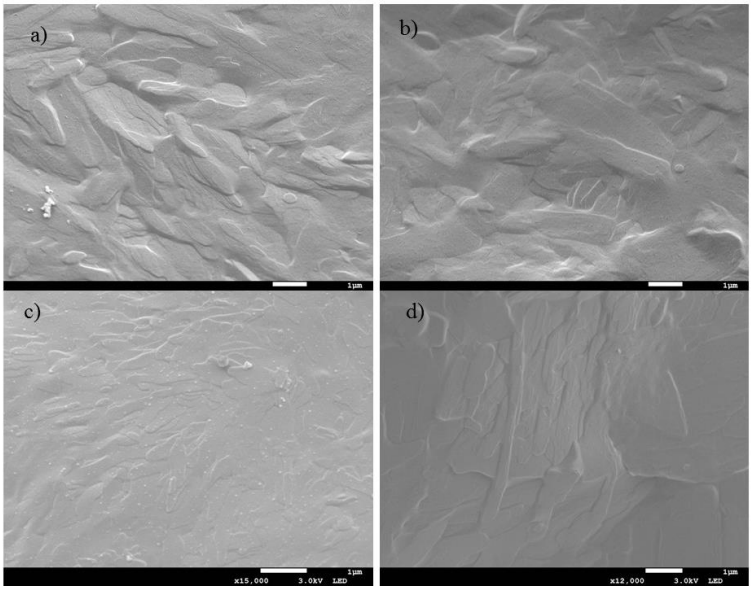

Figure 3.18 The structural building block of oleogels. The 10:0 (a), 9:1 (b), 8:2 (c), and 6:4 (d) distilled monoglycerides (DM):Phytosterols (PSs) oleogels have similar building block in plate-like shape of less than 1 μm in width.

In the bi-component oleogels, the structures exhibited some resemblance to that of DM mono-component gel suggesting that lamellar structures were still formed in those samples. However, in 6:4 oleogel, long needle-like crystals believed to be formed by PSs were also seen, which were absent in 9:1 and 8:2 oleogels. Overall, as evidenced from the electron microscopy images and supported by diffraction results, PSs starts to crystallize out as distinct entities once the ratio is increased beyond a threshold level (Bach & Wachtel, 2003).

In conclusion, mixed crystals can be observed in mixed component oleogels. These crystals seem to enhance the structural integrity of bi-component oleogels compared to the mono-component oleogels. This mixed crystal system agrees with the diffraction results discussed in section 3.4.2.3. The existence of impurities may influence the crystal habit (Smith et al., 2011), which was described previously (crystal defect). Besides that, DM and other non-ionic surfactants are often used as growth retarders for PSs (Christiansen et al., 2002; Engel & Schubert, 2005; Zychowski et al., 2016).

3.4.3 The role of phytosterols in influencing the growth of crystals and viscoelastic properties of monoglycerides gels

The use of MGs to structure edible liquid oil has been studied extensively, as an alternative to TAG structuring (Chen & Terentjev, 2009; Da Pieve et al., 2010; Da Pieve et al., 2011; Kesselman & Shimoni, 2007; Ojijo et al., 2004). However, the combination of MGs and PSs is relatively new. PSs alone could not structure edible oil, as the crystals are prone to contract and fail to form a network (Co & Marangoni, 2012). MGs do form firm gels but their aggregation during aging, which is related to the formation of a β-crystalline phase, compromises the oil binding properties (rheological properties) (Chen & Terentjev, 2009; Ojijo et al., 2004). Hence, controlling the crystallization behavior may control the aggregation (Chen & Terentjev, 2009; Ojijo et al., 2004). Several studies have therefore been performed to control the crystallization behavior of MGs either through combination with ethylcellulose (Lopez-Martinez et al., 2015) or shear nanocrystallization (Da Pieve et al., 2010).

It is interesting to note that the combination of DM with PSs has an influence on the crystallization, transition, rheology and morphology of the oleogels, as discussed in the previous sections. The PSs influence the crystallization and the transition of DM to a more stable polymorph. These effects are manifested in the non-isothermal and the

isothermal crystallization discussed in section 3.4.1.1 and 3.4.2.1. It has been reported that the gelation of DM molecules in oil involves intermolecular hydrogen bonding (Chen & Terentjev, 2009; Lupi, Greco, et al., 2016). Thus, it is likely that the effect of the PSs on the crystallization of DM is attributed to the ability of both molecules to interact via hydrogen bonding (Gater et al., 2013). The modified molecular assembly affected the microstructural development of bi-combination oleogels. The bi-component oleogels showed a slower elasticity development at the initial oscillatory time sweep. However, at the end of time sweep, the 9:1 and 8:2 (DM:PSs) oleogels outperformed the |G*| of DM mono-component. The enhanced rheological properties of the bi-component oloegels were confirmed with a frequency sweep on the stabilized oleogels after one-week stabilization.

Most likely a mixed crystal system is formed in the bi-component oleogels. This conclusion is based on the diffraction results obtained from both SAXD and WAXD, in which the corresponding diffraction peaks of DM and PSs appeared in the bi-component oleogels. As discussed in section 3.4.2.4, the PSs reduced the clustering of crystals and as such the physical properties of the bi-component oleogels improved. It has been discussed and explained that crystal defects may cause changes in the crystal morphology. Thus, crystal defects are the best explanation for how the intermolecular hydrogen bonding occurs between DM and PSs and how the condensing effect of the alkyl tail influences the crystal morphology of the bi-component oleogels (Smith et al., 2011; von Bonsdorff-Nikander et al., 2003). In section 3.4.2.4, small clustering was observed in the bi-component oleogels. In other words, a better spatial distribution of crystalline network in the presence of PSs, improves the viscoelastic properties (Marangoni & Rousseau, 1996; Tang & Marangoni, 2006a).

In oleogels, mixing two structurants has shown to affect the rheological properties of the bi-component oleogels (Bot et al., 2008). This effect is often due to modulation in the crystallization of host-structurant by additive/impurity. For instance, the interaction between SO and SA affected the crystallization and the morphology and thereby the hardness of the bi-component oleogel was higher (Blach et al., 2016). Additionally, Lopez-Martinez and co-workers studied the combination of monoglyceride and ethylcellulose and found the latter to delay transition from sub-α to β-polymorph. As a consequence, the interaction between monoglycerides and ethylcellulose improved

|G*| of oleogel (Lopez-Martinez et al., 2015). The combination of DM and PSs offers an interesting solution to the existing problems of crystal aggregation in DM as well as high-temperature melting of PSs. Interestingly, this combination is inspired by nature as sterols (phytosterols) are natural component of lipid membrane whilst DM has properties similar to phospholipids.

3.5 Conclusion

The cellular membrane is composed of sterols and phospholipids (polar lipid) as structuring units. We combined DM with PSs to investigate whether the effect observed in sterol-phospholipid mixtures can be exploited to structure sunflower oil. The 9:1 and 8:2 DM:PSs bi-component combinations structure sunflower oil better than the respective mono-component. In this comprehensive study, the improved structuring ability of the bi-component oleogels was attributed to the combined effect of hydrogen bonding and condensing effect of PSs on the crystallization and morphology of DM. Therefore, the bi-component oleogels formed a mixed crystal system with a modified microstructure, leading to enhanced rheological performance (Blach et al., 2016; Gravelle, Davidovich-Pinhas, Barbut, & Marangoni, 2017).

The thermal properties of DM in bi-component oleogels were affected by PSs. There was an observable delay in the crystallization and melting profiles of the bi-component oleogels, which was most pronounced in 6:4 DM:PSs. Consequently, the morphology of bi-component oleogels was affected, which could be due to intermolecular hydrogen bonding leading to crystal defects (Smith et al., 2011; von Bonsdorff-Nikander et al., 2003).

Phytosterols are functional material capable of reducing the blood cholesterol. The success of fabricating the oleogels based on PSs indirectly introduces this molecule into the human body. Conventionally, PSs are incorporated into a product only to improve the nutritional content, with less emphasis on its role as a structuring agent. This is partly because PSs have a high melting temperature which deter the quality of the end-product (gritty texture) (Acevedo & Franchetti, 2016; Co & Marangoni, 2012). In addition, high-temperature melting reduces its bioavailability, as it remains in crystal form at body temperature. In this chapter, on one hand, we have shown for the first time that DM is able to modulate the crystallization of PSs. On the other hand, PSs also influence the crystallization of DM and improve the viscoelasticity of DM-based

oleogels. Ultimately, the DM:PSs oleogel offers the food industry an alternative to a conventional structuring and a method to incorporate high-melting PSs into food products.

3.6 Perspectives

The resultant effect of the combination between DM and PSs has been clearly demonstrated in this chapter. However, the mechanism of PSs influencing the crystallization of DM requires additional analysis at molecular level. Thus, by using synchrotron radiation X-ray diffraction, the crystallization process of the oleogels was monitored during cooling and melting, and is reported in Chapter 5. Additionally, it is also recommended to complex monoglycerides with cholesterols to evaluate the effect of molecular structure on chain ordering.

Chapter 4 Mixed surfactant system of sucrose esters and lecithin as a synergistic approach for oil structuring

Relavant publication:

Bin Sintang, M.D., Danthine, S., Rimaux, T., Patel, A.R., Van de Walle, D., & Dewettinck, K. (2017). Mixed surfactant system of sucrose esters and lecithin as a synergistic approach for oil structuring. **Journal of Colloid and Interface Science**, 504, 387-396

4 Mixed surfactant system of sucrose esters and lecithin as a synergistic approach for oil structuring

4.1 Research strategy/hypothesis

This chapter researches the effect of sunflower lecithin (SFL) on the self-assembly of sucrose esters (SEs). It is hypothesized that by combining SFL with SE, aggregation could be prevented and gelation promoted (Nikiforidis & Scholten, 2014). The interesting self-assembly properties of surfactants, which are tuneable according to the shape of the molecules, solvent, and the presence of other surfactants, provides an alternative building block for oil structuring (Figure 4.1). Interestingly, the combination of surfactants with other amphiphilic molecules has been applied in pharmaceutical and chemical fields as a route to create functional architectures (Fong, Le, & Drummond, 2012; Vintiloiu & Leroux, 2008; Wang et al., 2012). Therefore, the hypothesis of this chapter is based on the ability of amphiphilic molecules to assemble into a different organization in response to a changing environment (bi-component). Moreover, the combination could eliminate the need for an organic polar solvent as a bridging agent, in which the solvent influences the bending angle and self-assembly (Bodennec et al., 2016; Hashizaki et al., 2009; Lan & Rogers, 2015; Rogers et al., 2008). Bridging agent is required to form stable microemulsions and mixed surfactant systems (Garti et al., 1999; Hashizaki et al., 2009; Nikiforidis & Scholten, 2014).

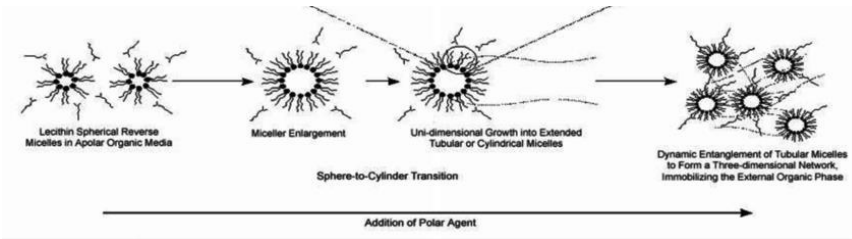

Figure 4.1 Schematic representation of self-assembly transition from spherical to cylindrical shape in lecithin added with polar component (Shchipunov, 2001).

4.2 Introduction

Amphiphilic compounds have been shown to structure in aqueous and organic media, producing both hydro- and oleogels with diverse properties. Amphilic structurants

induce gel formation through self-assembly by forming highly ordered structures (Sagalowicz, Leser, et al., 2006; Wang et al., 2012; Whitesides & Boncheva, 2002). Examples of these amphiphilic compounds are surfactants, which emerge as potential structurants due to their ability to self-assemble into different building blocks. These building blocks can be shaped as spherical micelles, cylindrical micelles, vesicles, and lamellar structures (Fong et al., 2012; Stubenrauch, 2001). In surfactants, the hydrophilic moieties interact through hydrogen bonding and the hydrophobic moieties interact via Van der Waals interaction. Their building block architectures rely upon both the properties of the hydrophilic region and those of the alkyl tails. These building block architectures are modelled by calculating the critical packing parameters (CPP) (Cardiel, Furusho, Skoglund, & Shen, 2015). Sugar ester-based surfactants (e.g. sucrose esters) are amphiphilic with various hydrophilic-lipophilic balance (HLB) values and fatty acid compositions, depending on their exact chemical structure.

Sucrose esters (SEs) are non-ionic surface-active surfactants, consisting of sucrose as the hydrophilic group and fatty acids as lipophilic group. The eight hydroxyl groups of sucrose can all be esterified with varying fatty acids resulting in sucrose monoester, sucrose diester, sucrose triester up to sucrose polyester, with different HLB values. Depending on their HLB, SEs are used as emulsifiers for either oil-in-water (O/W) or water-in-oil (W/O) emulsions. Moreover, SEs are used as solubilizing, foaming, anti-bacterial, releasing agents, and for the enhancement or inhibition of crystal growth in fats, and lubrication (Chansanroj & Betz, 2010; C. H. Chen, Zhang, Bi, & Cheong, 2015; Garbolino, Bartoccini, & Floter, 2005; Puppo, Martini, Hartel, & Herrera, 2002). SEs self-organize into micellar structures when dispersed in a solvent at particular concentrations and composition of SEs. At a concentration just above the critical micelle concentration (CMC) in an aqueous system, micelles are usually spherical in shape (Becerra, Toro, Zanocco, Lemp, & Gunther, 2008; Kawaguchi, Hamanaka, Kito, & Machida, 1991).

Microemulsions are isotropic and thermodynamically stable solutions, in which two immiscible liquids (such as water and oil), are brought together by means of an appropriate surfactant/co-surfactant mixture (Garti et al., 1999). Formation of sucrose ester microemulsions and their potential for food applications were studied by the groups of Garti and Fanun (Fanun, Wachtel, et al., 2001; Garti, Aserin, & Fanun, 2000; Garti, Clement, Fanun, & Leser, 2000; Garti et al., 1999). Sucrose esters alone fail to

form microemulsions (Fanun, Wachtel, et al., 2001; Garti et al., 1999). Sucrose monostearate in the presence of alcohol, water and medium chain triglyceride (MCT) does form water-in-oil microemulsions. These microemulsions are soft and waxy solid at room temperature, but liquefy above 40°C, which is interesting for food applications (Garti, Aserin, et al., 2000; Garti, Clement, et al., 2000). Microemulsions based on SEs and a co-surfactant exemplify the tuneable self-assembly of SEs. However, none of these studies reported the behavior of sucrose ester/co-surfactant mixture in edible oil without water/alcohol (polar solvent).

Dispersing SEs in a non-polar medium, such as sunflower oil is challenging because the hydrophilic monomers of SEs tend to aggregate. A procedure such as high-temperature mixing is not appropriate for all SEs because they exist in a broad HLB range. At high HLB, SEs are more hydrophilic and less compatible with non-polar solvents such as sunflower oil. Even at high temperature, hydrophilic SEs do not completely melt and usually tend to form aggregate (Szuts et al., 2007). For this reason, proper selection of SEs with an appropriate HLB value is necessary for oil structuring.

Another approach to achieve oil structuring with SEs is to create a mixed surfactant system using SEs and a second surfactant (Arleth et al., 2003; Kwon & Kim, 2001; Rodriguez-Abreu et al., 2005; Schaink et al., 2007; Sharma, Acharya, & Aramaki, 2007; Sharma, Rodriguez-Abreu, Shrestha, & Aramaki, 2007a). Mixed surfactant systems have been shown to induce and enhance gelation of organic solvents, which is of great interest in chemical and pharmaceutical industries (Rodriguez-Abreu et al., 2005). Commonly, one surfactant influences the self-assembly of the other surfactant which is accompanied with a change in phase behavior (Acharya, Varade, & Aramaki, 2007; Engelskirchen, Acharya, Garcia-Roman, & Kunieda, 2006; Sharma, Acharya, et al., 2007; Sharma, Rodriguez-Abreu, et al., 2007a; Sharma, Rodriguez-Abreu, Shrestha, & Aramaki, 2007b) and enhanced interfacial properties. For example, the combination of lecithin and sucrose monoesters (HLB12) enhanced the viscosity of mixture by forming reverse worm-like micelles (Hashizaki et al., 2009).

Currently, a wide selection of surfactants is available that can be used as a co-surfactant. Co-surfactants should be edible and soluble in the dispersing medium. Lecithin has shown to influence the self-assembly of host molecules, leading to

gelation. This effect has been observed in combination systems with sorbitan tristearate (Pernetti, van Malssen, Kalnin, et al., 2007) and α-tocopherol (Nikiforidis & Scholten, 2014). Co-surfactants are believed to affect the hydrogen bonding of the available hydroxyl groups between the host surfactant molecules, which influences their self-assembly properties. The additive surfactant stabilizes the system by bridging the host surfactant together through hydrogen bonding, preventing host aggregation (Bodennec et al., 2016; Hashizaki et al., 2009). The polar solvent (e.g. water and alcohol) causes micellar swelling and induces one-dimensional growth of reverse micelles that transfer into polymer-like micelles (Bodennec et al., 2016; Kwon & Kim, 2001; Nikiforidis & Scholten, 2014; Rodriguez-Abreu et al., 2005; Sharma, Rodriguez-Abreu, et al., 2007a; Shchipunov et al., 1999). The success of mixed surfactant systems is a possible route to tune oleogel properties which can increase the application of oleogels in food and drug delivery.

Although extensive research has been performed on tuning the assembly of SEs to wormlike micelle in mixed surfactant system (Bolzinger-Thevenin, Grossiord, & Poelman, 1999; Rodriguez, Acharya, Hinata, Ishitobi, & Kunieda, 2003), limited research can be found on its potential as a structurant and behavior in the presence of a co-surfactant in sunflower oil. Therefore, in this chapter the feasibility of SEs as a structurant and the effect of SFL as co-surfactant in a mixed surfactant system were investigated.

4.3 Materials and methods

4.3.1 Materials

Sucrose esters (HLB-2), stearic acid-based (approximate composition: sucrose monoester (±11%), sucrose diesters (±15%), sucrose triester (±16%) and sucrose polyesters (±58%), was supplied by Sisterna B.V, Netherlands. Refined sunflower oil and sunflower lecithin were supplied by Vandemoortele Lipids N.V., Belgium.

4.3.2 Oleogels preparation

Oleogels were prepared with SEs, SFL, and their combinations in ratios of 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 0:10 (wt%) in sunflower oil. Separately, the structurants and sunflower oil were heated to 90°C prior to mixing under continuous stirring for approximately 20-30 minutes using a magnetic stirrer (Model EM3300T,

Labotech Inc., Germany). The mixture was then cooled to room temperature during 10 minutes before being transferred to fridge. The samples were stored at 5°C until further use.

4.3.3 Thermal behavior

Details of the instrument deployed in this chapter are described in Chapter 2. In Chapter 2, the preparation steps of the DSC pan are also described in detail.

The oleogels of SEs and its combinations with SFL prepared at total structurant of 10 wt% were heated to 100°C and held for 10 min to eliminate the crystal history. Subsequently, the samples were cooled to 5°C at a cooling rate of 10°C/min. The samples were then kept isothermal for 1 min before heating to 100°C at a heating rate of 5°C/min.

The effect of crystallization period on the melting behavior of selected oleogels was investigated by subjecting the oleogels to isothermal crystallization for three hours. Then, the oleogels were heated to 100°C at a heating rate of 5°C. Thereafter, the same pans were immediately transferred to the fridge at 5°C, and stored for at least one week. After one week, the pans were again heated from 5°C to 100°C at a heating rate of 5°C/min.

4.3.4 Small amplitude oscillatory stress

The instrument used for this analysis is described in Chapter 2

4.3.4.1 Oscillatory time sweep

The procedure is comprehensively explained in Chapter 2.

4.3.4.2 Amplitude stress sweep

The procedure is comprehensively explained in Chapter 2.

4.3.4.3 Frequency sweep

The procedure is comprehensively explained in Chapter 3.

4.3.5 Optical light microscopy

The procedure is comprehensively explained in Chapter 2.

4.3.6 Scanning electron microscopy

The procedure is comprehensively explained in Chapter 2.

4.3.7 Confocal laser scanning microscopy

The microstructure of the oleogels was visualized using Confocal Laser Scanning Microscopy (CLSM). The SEs were stained with rhodamine 6g by dispersing the dye in the molten SEs prior to the addition of sunflower oil. The gel was prepared using the same procedure as described above. A small amount of oleogel was smeared onto a glass slide and covered with cover slid.

The instrument and software used for this analysis are described in Chapter 2.

4.3.8 Powder x-ray diffraction

The procedure is comprehensively explained in Chapter 2.

4.3.9 Statistical analysis

The procedure is comprehensively explained in Chapter 2.

4.4 Results and discussion

4.4.1 Mixing behavior of sucrose esters and lecithin

Eleven oleogels were prepared using SEs, SFL and their combinations, namely 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 0:10 SEs:SFL in sunflower oil. The SEs and SFL mono-component oleogels at 10 wt% did not solidify, though the oleogels were stored at 5°C for one week. At room temperature, the oleogels prepared from combinations of 9:1 to 6:4 SEs:SFL maintained solid-like properties (Figure 4.2). The SEs:SFL ratios ranging between 5:5 to 3:7 started to flow when left at room temperature for 10 minutes. When the oleogels were stored at 5°C, solid-like properties were also percieved at SEs:SFL ratio 3:7.

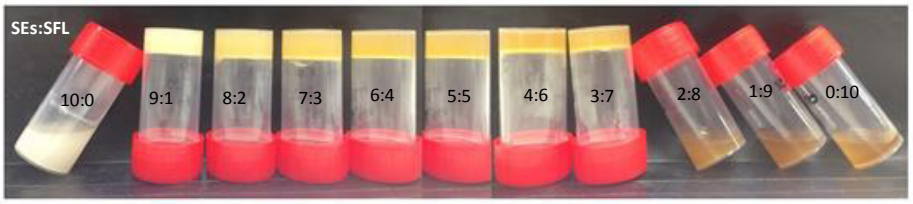

Figure 4.2 The series of oleogels prepared with a combination of sucrose esters (SEs) and sunflower lecithin (SFL) at different ratio at 10 wt% total concentration in sunflower oil.

To further characterize the oleogels, their mixing behavior was studied using differential scanning calorimetry. The crystallization and melting behavior were investigated to elucidate the change in the structural formation of the oleogels. The same technique was applied to investigate the effect of intermolecular hydrogen bonding in 12-hydroxystearic acid (Lan & Rogers, 2015) and oleic acid-sodium oleate (Nikiforidis et al., 2015) oleogels. During cooling, the shape of the crystallization peak changed with the change in SEs:SFL ratio (Figure 4.3). Decreasing the SEs content shifted the onset and peak crystallization to significantly lower temperatures (Table 4.1). The shape of the peak sharpened as the concentration of SEs decreased (Figure 4.3). The 10:0, 9:1, and 8:2 oleogels displayed a peak with broad shoulder prior to the main crystallization peak. In contrast, only one sharp peak was observed for the 7:3, 6:4, 5:5, 4:6, and 3:7 SEs:SFL oleogels. The 2:8 and 1:9 oleogels exhibited a small crystallization peak, while 0:10 produced no identifiable crystallization peak (Figure 4.3). Therefore, we can deduce that the crystallization peak is ascribed to the SEs while the SFL exists as a modifier (Delacharlerie et al., 2016), since the SFL mono-component yielded no crystallization peak during cooling (Figure 4.3). This result agrees with the image in Figure 4.2.

The enthalpy of crystallization was significantly decreased with the change of ratio, especially between 10:0 and the ratios of 9:1, 8:2, and 7:3 SEs:SFL (Table 4.1). However, there was no linear correlation between the enthalpy of crystallization and the change in the SEs ratio. This result signifies the formation of structure resulted from the combination (changing the environment) of SEs and SFL, leading to the observed change in enthalpy (Gao, Wu, Emge, & Rogers, 2013; Nikiforidis et al., 2015).

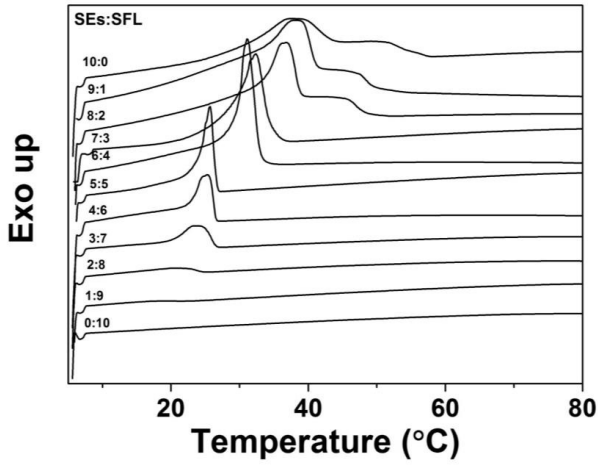

Figure 4.3 Crystallization (cooling) profile of the sucrose esters (SEs):sunflower lecithin (SFL) combination oleogels. The oleogels were crystallized with cooling rate of 10°C/min to 5°C.

Table 4.1 The onset temperature (T_{on}), peak temperature (T_{cry}), and enthalpy (H_{cry}) during crystallization of the sucrose esters (SEs):sunflower lecithin (SFL) combination oleogels (N=3 of independent sample).

Oleogels (SEs:SFL)	T_{on} (°C)	T_{cry} (°C)	H_{cry}(J/g)
10:0	57.43±0.30$^{h\ (h)}$	37.11±0.21$^{i\ (i)}$	9.28±0.46$^{f\ (f)}$
9:1	54.79±0.15$^{g\ (g)}$	38.02±0.02$^{j\ (h)}$	7.65±0.38$^{e\ (e)}$
8:2	47.72±0.94$^{f\ (f)}$	36.27±0.19$^{h\ (g)}$	7.39±0.29$^{e\ (e)}$
7:3	36.68±0.02$^{e\ (e)}$	34.68±0.41$^{g\ (f)}$	7.14±0.56$^{e\ (e)}$
6:4	34.43±1.66$^{d\ (d)}$	31.24±0.76$^{f\ (e)}$	5.31±0.32$^{d\ (d)}$
5:5	27.33±0.77$^{c\ (c)}$	25.90±0.43$^{e\ (d)}$	3.02±0.17$^{c\ (c)}$
4:6	26.53±0.87$^{c\ (c)}$	25.02±0.36$^{d\ (c)}$	2.53±0.25$^{c\ (c)}$
3:7	26.43±0.25$^{c\ (c)}$	23.19±0.08$^{c\ (b)}$	1.48±0.15$^{b\ (b)}$
2:8	24.14±0.16$^{b\ (b)}$	19.52±0.27$^{b\ (a)}$	0.41±0.08$^{a\ (a)}$
1:9	20.00±0.49$^{a\ (a)}$	18.80±0.12$^{a\ (a)}$	ND
0:10	ND	ND	ND

Values indicated with different letters in the same column are significantly different ($P < 0.05$)
Values indicated with different letters, inside a bracket, in the same column are significantly different ($P < 0.01$)
ND = non-detected

The melting behavior of the oleogels was obtained immediately after cooling, and is illustrated in Figure 4.4 (Table 4.2). The decrease in melting temperature of oleogels was directly proportional to the decrease in the SEs ratio, similar to their crystallization

behavior. At low SEs concentrations, low melting temperatures were observed, wherein at high SEs concentrations, the oleogels melted at high temperature. The oleogels with decreasing SEs concentrations melted at temperature(T_{mp}) significantly lower than the SEs mono-component (Figure 4.4 and Table 4.2).

The 0:10 SEs:SFL oleogel exhibited no melting peak, which was similar with the crystallization profile. Similarly, there were no observable crystallization and melting peak of SFL mono-component reported in literature which supports our finding (Pernetti, van Malssen, Kalnin, et al., 2007). Meanwhile, the melting enthalpy was significantly decreased with the change of SEs' ratio, which is similar to the observed crystallization enthalpy.

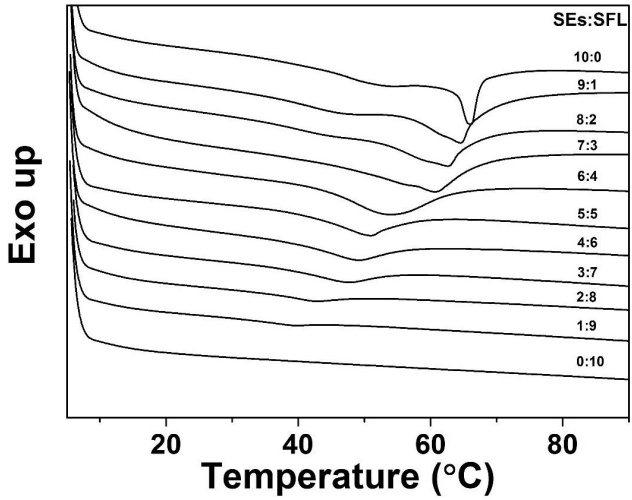

Figure 4.4 Melting profile of the sucrose esters (SEs):sunflower lecithin (SFL) combination oleogels measured immediately after cooling to 5°C. The oleogels were heated with heating rate of 5°C/min.

Table 4.2 The peak melting (T_{mp}), enthalpy (H_{cry}), and offset temperature (T_{off}), during melting of the sucrose esters (SEs):sunflower lecithin (SFL) combination oleogels (N=3 of independent sample).

Oleogels (SEs:SFL)	T_{mp} (°C)	H_{mp}(J/g)	T_{off} (°C)
10:0	65.81±0.08$^{i\,(g)}$	7.48±0.05$^{i\,(g)}$	72.82±0.45$^{g(f)}$
9:1	64.68±0.09$^{h\,(g)}$	6.89±0.31$^{h\,(f)}$	73.76±0.25$^{g(f)}$
8:2	60.16±2.02$^{g\,(f)}$	6.49±0.16$^{g\,(e)}$	73.57±0.42$^{g(f)}$
7:3	60.30±0.31$^{g\,(f)}$	6.19±0.27$^{f\,(e)}$	73.19±0.44$^{g(f)}$
6:4	53.07±0.18$^{f\,(e)}$	4.77±0.16$^{e\,(d)}$	67.68±0.94$^{f(e)}$
5:5	50.46±0.18$^{e\,(d)}$	2.70±0.18$^{d\,(c)}$	60.45±0.24$^{e(d)}$
4:6	49.06±0.74$^{d\,(d)}$	2.35±0.23$^{c\,(c)}$	60.05±0.76$^{d(d)}$
3:7	46.20±0.17$^{c\,(c)}$	1.41±0.06$^{b\,(b)}$	55.90±0.85$^{c(c)}$
2:8	41.50±0.14$^{b\,(b)}$	0.53±0.06$^{a\,(a)}$	47.74±0.96$^{b(b)}$
1:9	39.79±0.22$^{a\,(a)}$	ND	41.54±0.22$^{a(a)}$
0:10	ND	ND	ND

Values indicated with different letters in the same column are significantly different ($P < 0.05$)
Values indicated with different letters, inside a bracket, in the same column are significantly different ($P < 0.01$)
ND = non-detected

4.4.1.1 Melting behavior of oleogels stabilized at different periods

Monitoring the melting behavior of oleogels for different crystallization periods gives an indication on the possible change in their structure (Foubert et al., 2008; Verstringe et al., 2013). The melting behavior of selected oleogels of with ratios 10:0, 8:2, 7:3, and 6:4 SEs:SFL were analyzed after three-hour and one-week stabilization at 5°C. As shown in Figure 4.5 and summarized in Table 4.3, the 8:2 and 7:3 SEs:SFL oleogels exhibited a change in peak temperature, but not in the melting range. Meanwhile, the 10:0 and 6:4 SEs:SFL oleogels exhibited no change in the melting profile between three-hour and one-week stored oleogels. Overall, the melting enthalpy was slightly increased after one-week stabilization, with the 10:0 and 8:2 SEs:SFL increased significantly.

Table 4.3 The peak melting temperatures (T_{mp}), melting enthalpy (H_{mp}), and offset melting temperature (T_{off}) of the sucrose esters (SEs):sunflower lecithin (SFL) combination oleogels at different crystallization periods (t-test) (N=3 of independent samples).

Oleogels (SEs:SFL)	Three-hour			One-week		
	T_{mp} (°C)	H_{mp} (J/g)	T_{off} (°C)	T_{mp} (°C)	H_{mp} (J/g)	T_{off} (°C)
10:0	65.82±0.06^{dA}	7.28 ±0.15^{cA}	73.16 ±2.15^{cA}	65.01 ±0.56^{cA}	8.38 ±0.49^{dB}	74.29 ±0.52^{dA}
8:2	61.89 ±0.23^{cA}	6.87 ±0.25^{cA}	73.70 ±0.71^{cA}	53.45 ±0.34^{bB}	7.39 ±0.13^{cB}	72.68 ±1.06^{cA}
7:3	60.17 ±0.23^{bA}	6.18 ±1.06^{bA}	70.31 ±0.40^{bA}	53.43 ±0.07^{bB}	6.09 ±0.04^{bA}	68.40 ±0.49^{bB}
6:4	53.56 ±0.96^{aA}	4.54 ±0.37^{aA}	65.64 ±0.70^{aA}	52.12 ±0.16^{aA}	5.12 ±0.37^{aA}	65.20 ±0.81^{aA}

Small letter = indicative the significant difference (P<0.05) between the oleogels in the same colum
Capital letter= Indicative the significant difference (P<0.05) of the same oleogel on the same parameter at different crystallization period (Row)

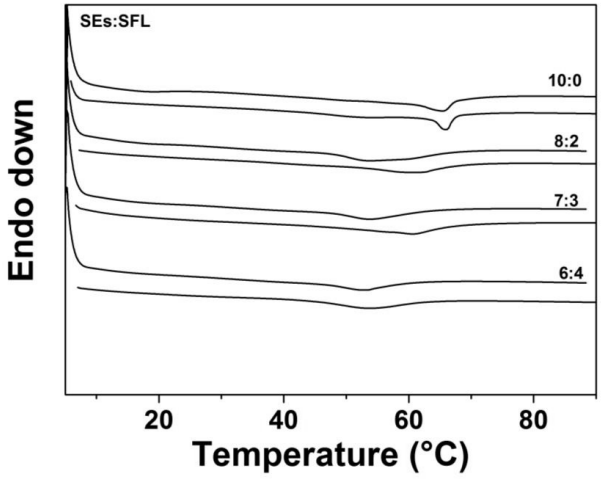

Figure 4.5 The melting profile of the sucrose esters (SEs):sunflower lecithin (SFL) combination oleogels stabilized for three-hour (bottom) and one-week (top) storage at 5°C.

Based on the thermal behavior presented above, the change in composition significantly affected both the crystallization and melting behavior of the oleogels. The observed effect of SFL addition indicates a change in the self-assembly of SEs. Lan and Rogers observed that the type of solvent influences the intramolecular hydrogen bonding between 12-hydroxystearic acid, influencing the melting behavior of oleogels (Lan & Rogers, 2015). The solvent becomes a bridge between the 12-hydroxy stearic acid molecules and induces the formation of tubular structure. In more polar solvents, extensive hydrogen bonding between solvent-gelator disrupted the growth of one-dimensional crystals (Gao et al., 2013; Lan & Rogers, 2015; Rogers et al., 2008). Thus, hydrogen bonding can influence the molecular assembly and as such the thermal behavior. The observed changes in the thermal behavior and the self-assembly can

be the result of hydrogen bonding between SFL and SEs (Hashizaki et al., 2009; Kwon & Kim, 2001; Nikiforidis & Scholten, 2014; Ullrich, Metz, & Mader, 2008).

4.4.2 Rheological behavior of oleogels

The microstructural development is an important attribute to evaluate the oil-structuring properties of structurants. The gelation of SEs and the effect of SFL were assessed based on the evolution in the complex modulus (|G*|) and phase angle (δ) as a function of temperature and time (Lupi, Greco, et al., 2016). Figure 4.6 shows the graph of |G*| and δ during cooling from 90 to 5°C and a three-hour isothermal period at 5°C (Figure 4.7).

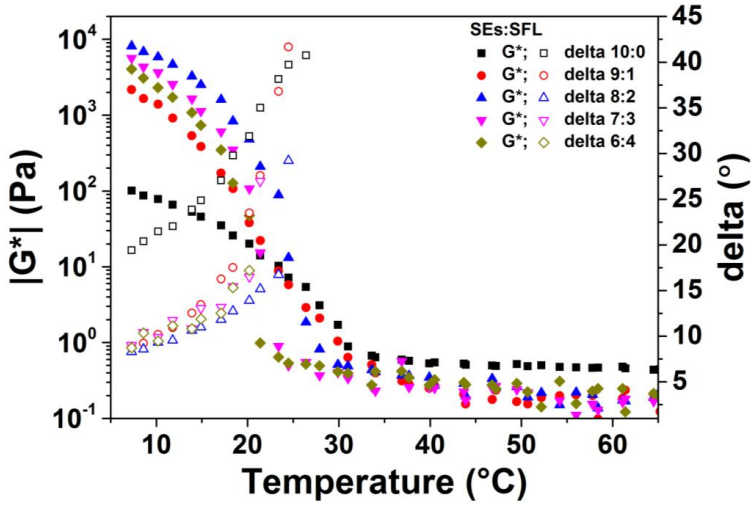

Figure 4.6 The evolution on the complex modulus and phase angle of the sucrose esters (SEs) and sunflower lecithin (SFL) combination oleogels subjected to cooling from 90°C to 5°C with cooling rate of 10°C/min.

Based on Figure 4.6 and Table 4.4, the 10:0 oleogels gelled at a higher temperature, followed by 9:1, 8:2, 7:3, and 6:4 SEs:SFL. Although 7:3 and 6:4 SEs:SFL exhibited significantly low gelation temperature, their |G*| steeply increased as the temperature approached 5°C (Figure 4.6). Moreover, the gelation point (Figure 4.6) was observed after the peak crystallization temperature (Figure 4.3 and Table 4.4), which was also observed by Lupi and co-workers (Lupi, Greco, et al., 2016). This phenomenon is related to the type of non-covalent interaction dominating the self-assembly formation.

Table 4.4 The gelling temperature (T_{gel}) of the sucrose esters (SEs) and sunflower lecithin (SFL) combination oleogels during temperature ramp (N=3 of same oleogel)

Oleogels (SEs:SFL)	T_{gel} (°C)
10:0	31.00±2.17$^{c\ (d)}$
9:1	26.07±0.31$^{b\ (c)}$
8:2	25.27±0.32$^{b\ (bc)}$
7:3	22.43±0.85$^{a\ (ab)}$
6:4	21.60±1.41$^{a\ (a)}$

Values indicated with different letters are significantly different (P < 0.05)
Values indicated with different letters, inside a bracket, are significantly different (P < 0.01)

Based on Figure 4.7 and Table 4.5, the bi-component oleogels showed higher |G*| than the mono-component oleogel of SEs. This result agrees with the results presented in Figure 4.2, which shows the SEs mono-component oleogel exist as structured fluid. The 7:3 SEs:SFL oleogel recorded the highest |G*|, while the SEs mono-component recorded the lowest |G*|. The increase in |G*| was influenced by the addition of SFL and reached a maximum at the ratio 7:3 SEs:SFL. The increased |G*| observed in the bi-component oleogels might be related to the effect of SFL on the microstructural development of the oleogel, as was also demonstrated by Delacharlerie et al. (Delacharlerie et al., 2016).

Table 4.5 The phase angle (δ) and complex modulus |G*| values of the sucrose esters (SEs) and sunflower lecithin (SFL) combination oleogels at the end of three-hour time-sweep (N=3 of same oleogel).

Oleogels (SEs:SFL)	Phase angle/Delta (°)	Complex modulus |G*| (kPa)
10:0	6.95±0.36$^{c\ (b)}$	0.53±0.04$^{a\ (a)}$
9:1	3.97±0.03$^{ab\ (a)}$	10.84±1.29$^{b\ (b)}$
8:2	4.17±0.25$^{b\ (a)}$	31.87±1.85$^{c\ (c)}$
7:3	3.77±0.08$^{ab\ (a)}$	37.62±1.50$^{d\ (d)}$
6:4	3.70±0.26$^{a\ (a)}$	29.81±0.68$^{c\ (c)}$

Values indicated with different letters in the same column are significantly different (P < 0.05)
Values indicated with different letters, inside a bracket, in the same column are significantly different (P < 0.01)

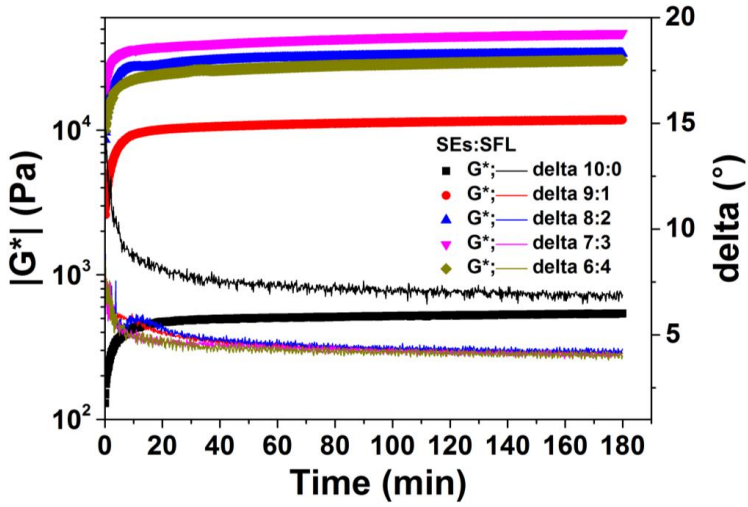

Figure 4.7. The evolution in the complex modulus and phase angle during oscillatory time-sweep at 5°C of the sucrose esters (SEs) and sunflower lecithin (SFL) combination oleogels. The measurement was consequtively performed after the temperature ramp.

The rheological results suggest that the modification in self-assembly leads to solidification, measured with |G*|. Moreover, the 7:3 and 6:4 SEs:SFL ratios showed low δ values, indicative of a higher oleogel strength (Table 4.5). The bi-component oleogels of SEs:SFL show promising physical properties. This promising viscoelastic behavior of SEs has been studied in the pharmaceutical field due to its ability to assume different molecular ordering, which is capable to act as a drug carrier (Rodriguez-Abreu et al., 2005; Szuts, Budai-Szucs, Eros, Otomo, & Szabo-Revesz, 2010; Ullrich et al., 2008).

The oleogels with combinations of 9:1, 8:2, 7:3, and 6:4 SEs:SFL were selected for further investigation of their mechanical strength. We conducted amplitude stress sweeps and frequency sweeps (FS) which express the strength in term of storage modulus (G') and loss modulus (G"). In amplitude stress sweeps, the oleogels were subjected to an increasing stress in which the linear region (LVR) is defined as the region where the G' and G" values are independent of oscillatory stress (Mezger, 2011). Again, increasing the SFL in the bi-component oleogels resulted in a higher G' (Figure 4.8).

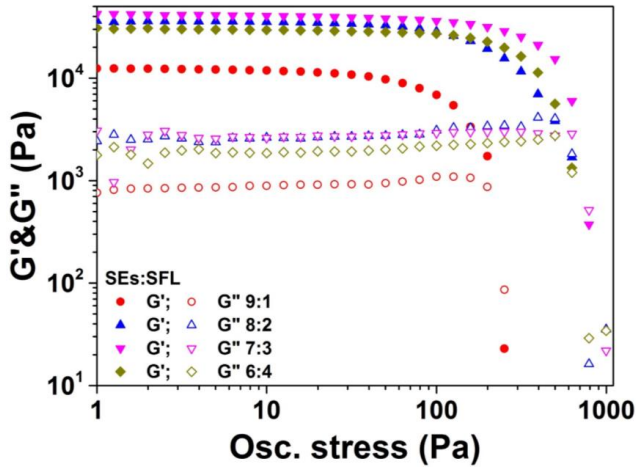

Figure 4.8. The amplitude stress sweep of the sucrose esters (SEs) and sunflower lecithin (SFL) combination oleogels measured continuously after the three-hour time sweep at 5°C

At the ratio 6:4, the G' decreased to values even lower than the ones of 8:2 ratio SEs:SFL, which shows that the physical properties of the oleogels were also affected by the concentration of SEs (Szuts et al., 2010). The SEs provides the solid particles for the building blocks which are required to sustain the properties of oleogel (Szuts et al., 2010). SFL influences the assembly of the SEs building blocks, which is also known to influence the crystallization (Delacharlerie et al., 2016). The physical properties of the oleogels were therefore governed by the balance between the SEs and SFL, as observed in the 7:3 SEs:SFL oleogel, which is stronger than the other oleogels. In 6:4 SEs:SFL, the composition of SEs was reduced which mean less solid particles for building blocks. This resulted in formation of oleogel with low G' but with extended LVR (better resistance towards oscillatory stress). In 8:2 SEs:SFL, contrary, there were more solid particles available with less SFL which created brittle oleogel (less resistance towards stress).

The time-dependency of oleogels was studied by means of frequency sweep (Figure 4.9), after one-week stabilization. Frequency sweep test enables structural delineation of gelling systems as a function of time. When storage and loss modulus are plotted as a function of frequency, strong gels show a horizontal line on the graph (i.e. frequency independence), whereas weak gels show a positive line with moduli values increasing as a function of the rate of deformation (frequency dependence) (Doan et

al., 2015; Mezger, 2011; Patel, Babaahmadi, Lesaffer, & Dewettinck, 2015). In addition, frequency sweeps explain the non-covalent interactions between the particles that structure the system (oleogel). Due to the nature of the non-covalent interactions, the transient network (stabilized by the weak interactions) forms and breaks reversibly during measuring (Mezger, 2011).

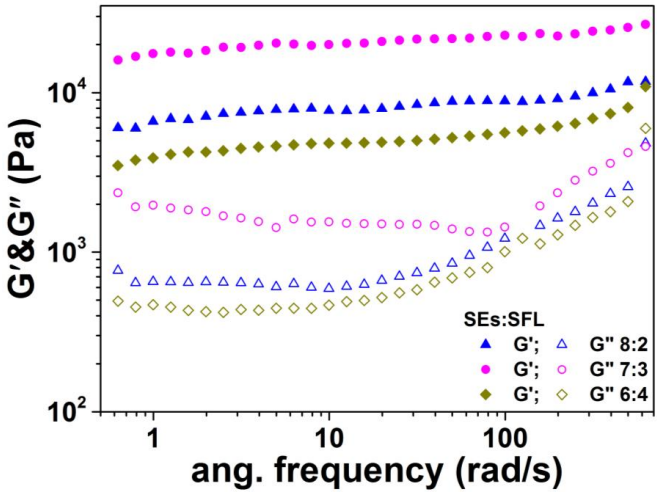

Figure 4.9. The frequency sweep pattern of the sucrose esters (SEs) and sunflower lecithin (SFL) combination oleogels performed after one-week stabilization at 5°C.

In agreement with the previous test, higher G' was found in the bi-component oleogel with 7:3 ratio SEs:SFL (Figure 4.9). For the other ratios, similar trends as in the LVR test were discerned, with the G' of the 8:2 ratio higher than the 6:4 ratio. As the angular frequency increased, all the oleogels showed slight increase in G' as well, which is characteristic of a weak gel (Mezger, 2011) (Figure 4.9). Moreover, the 7:3 bi-component oleogel showed less dependency towards frequency compared to the other ratios. The rheological results imply that the improved structural formation of the 7:3 ratio could be due to intermolecular non-covalent interactions. As observed in the amplitude sweep test, we can deduce an interdependent relationship between SEs and SFL in structuring the oil, as explained in the amplitude sweep. The change in the rheological properties is also supported with the observed change in the thermal behavior, especially on the shape of crystallization peak.

The tubular micelles conformation in the mixed surfactant system induces an increase in the viscosity of solution upon the initial addition of the co-surfactant. Then, the viscosity decreases as more co-surfactant is added to the host-surfactant mixture due to branching of tubules/disrupting the growth of micelles (Acharya et al., 2007; Cardiel et al., 2015; Rodriguez-Abreu et al., 2005; Szuts et al., 2010). In addition, oleogels structured through the entanglement of tubules of self-assembled molecules, also show interdependency between the ratio of the combination and the rheological properties, due to branching because of crystallization mismatch (Bodennec et al., 2016; Lan & Rogers, 2015; Nikiforidis et al., 2015; Nikiforidis & Scholten, 2014). Thus, a plausible explanation on the change of modulus (Fig. 4.6, 4.7, 4.8, and 4.9) can be the further modulation of the intermolecular interaction, affecting the self-assembly and the morphology.

Rheological properties of SEs:SFL oleogel at 7:3 is comparable to oleogels prepared with different structurants (Bodennec et al., 2016; Hashizaki et al., 2009; Nikiforidis et al., 2015; Rogers et al., 2008). As described in the oscillatory results, our studied ratios had higher modulus values (Figure 4.6, 4.7, 4.8, and 4.9) than 20 wt% lecithin-based oleogel (Bodennec et al., 2016), and comparable modulus values to those of the 5 wt% 12-hydroxystearic acid oleogels (Rogers et al., 2008), respectively. In most of those studies, the modulation in the rheological properties was attributed to the change in intermolecular interaction. The interaction arises from the change in the environment factors such as type of solvent and added co-structurants (Fong et al., 2012). Similarly, the observed changes in the thermal behavior agree with the rheology results thus, support the hypothesis of the modulation in self-assembly (Lan, Corradini, Weiss, Raghavan, & Rogers, 2015). Despite the decrease in both temperature and enthalpy, the bi-component oleogels showed improved viscoelastic properties than the SEs mono-component oleogel.

4.4.3 Morphology of oleogels

The microstructure of oleogels after one-week stabilization at 5°C (Figure 4.10 and 4.11) was visualized using optical microscopy in both normal and polarized light function. The visualization only considered the bottom layer, since phase separation was observed in the SE mono-component (Figure 4.10a, 4.11a and 4.12a). The bi-component oleogels showed more fine and dense structural network compared to the

mono-component system (Figure 4.10b-d and 4.11b-d). Although a detailed modification could not be clearly discerned, a dense structural network demonstrates the role played by SFL in the bi-component oleogels. The different morphologies observed in the SEs mono-component and the bi-component oleogels support the results on thermal and rheological properties discussed previously.

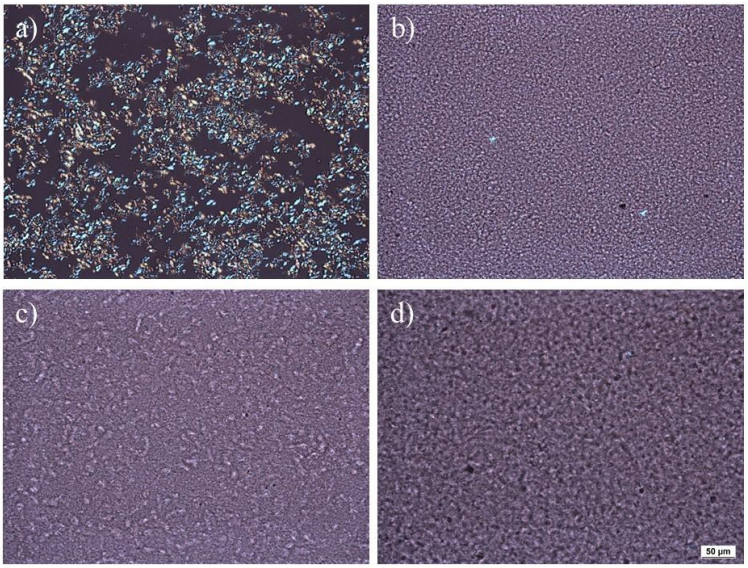

Figure 4.10 The microstructure of 10:0 (a), 8:2 (b), 7:3 (c), and 6:4 (d) sucrose esters (SEs):sunflower lecithin (SFL) oleogels visualized under the polarized light microscope. All the images have the same scale bar (50µm).

As demonstrated in the microscopic images, the bi-component oleogels have a different morphology compared to the mono component oleogel (Figure 4.10a and 4.11a). The scanning electron microscopy was used to visualize the microstructure of oleogels in more details. This allows identification of the differences between the mono-component and bi-component oleogels at microstructural level. The SEs mono-component oleogel revealed globular or spherical structures (Figure 4.11a). The size of the structure was 10µm or smaller and had a polydisperse distribution. This polydisperse globular structure explains the inability of solely SEs to structure the sunflower oil. These globular structures tend to aggregate and thus, fail to form a network required for structuring, as was discussed in the rheological section. A better

spatial distribution usually leads to better network formation (Bin Sintang et al., 2017; Kouzounis et al., 2017; Lan & Rogers, 2015; Marangoni & Rousseau, 1996; Nikiforidis et al., 2015). In the SEs:SFL combination oleogels (Figure 4.11b-d), a more uniform and dispersed network were seen indicating the effect of SFL in the gelation. In other word, the SFL inhibits the SFL molecules to enclose and forming globular structure.

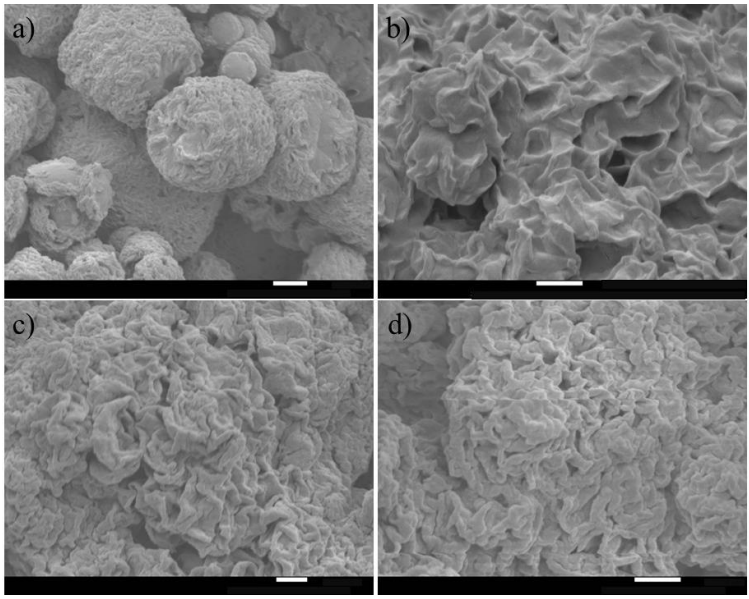

Figure 4.11 The microstructure of 10:0 (a), 8:2 (b), 7:3 (c), and 6:4 (d) sucrose esters (SEs):sunflower lecithin (SFL) oleogels visualized under the Cryo-Scanning Electron Microscope. All the oleogels were subjected to oil extraction procedures to optimize the visualization (scale bar = 1μm).

To elucidate the morphology of the selected oleogels, confocal microscope was used for the purpose of visualization, using rhodamine 6G as the dye. This fluorescent dye has shown to physically adsorb to sucrose esters and phosphorylcholine, which was used to localize the molecules in plant tissues (Lin & Wagner, 1994) and polymer-based biomaterials (Wang et al., 2005). The labelling mechanism is due to the amphiphilic properties of those component that have affinity thus, physically attach to the dye. Figure 4.12 shows fluorescent and brightfield images of 10:0 and 7:3 SEs:SFL oleogels.

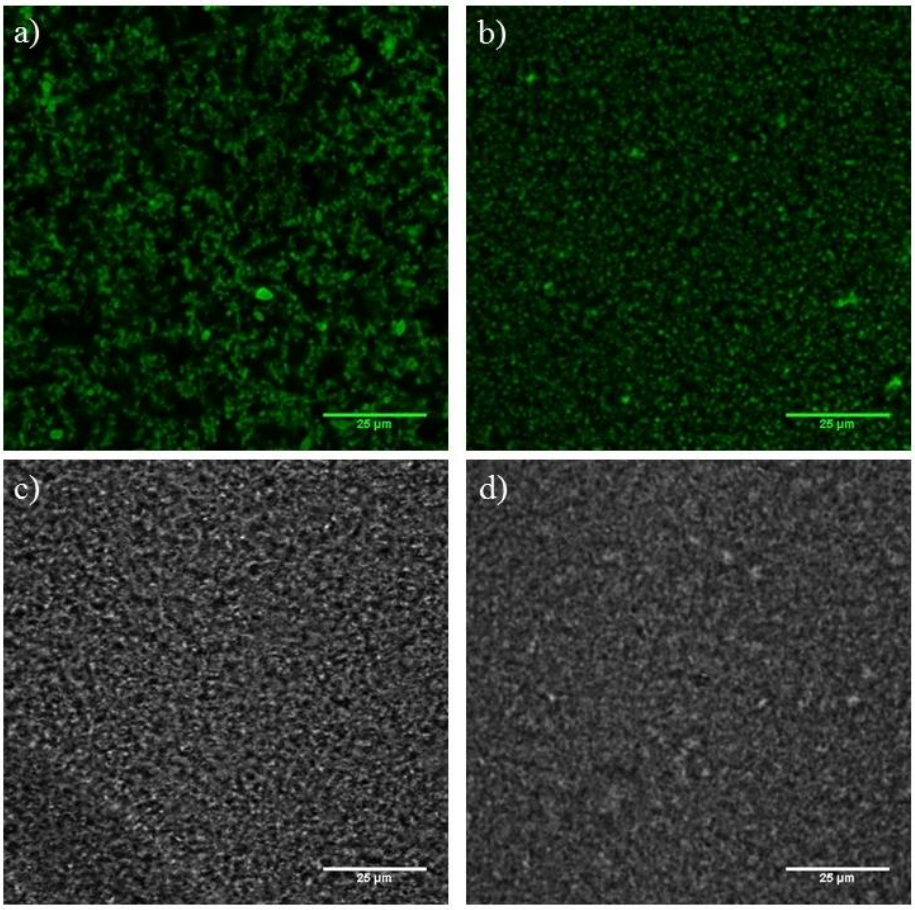

Figure 4.12 The microstructure of 10:0 (a and c) and 7:3 (b and d) sucrose esters (SEs):sunflower lecithin (SFL) oleogels visualized under the Confocal Laser Scanning Microscope (from one plane). The a and b images represent the fluorescent, while the c and d images represent the brightfield. Rhodamine 6G was used as the dye (scale bar = 25 µm).

The dark (black) background seen in the fluorescent images represents the empty space filled by sunflower oil (unstained) since, the sunflower oil was not subjected to any fluorescent dye. The 10:0 SEs:SFL oleogel exhibited globular structures, consistent with the morphology observed in electron microscope (Figure 4.11a and c). The large globular structures disappeared in the 7:3 SEs:SFL oleogel, which consisted of more fine and uniform structures. These images in Figure 4.12 provide additional evidence that the bi-component oleogels underwent a change in self-assembly.

The structural network of the bi-component oleogels is uniform and dense, as illustrated in Figure 4.10 b, c, and d. The network formed by these structures is consistently similar in all studied bi-component oleogels (Figure 4.10). Moreover, the surface of the microstructure resembles similar pattern in mono-component and bi-component oleogels (Figure 4.11). Therefore, we hypothesized that SFL hinders the SEs molecules to enclose into globular structures, but instead a dense network is formed. This hindrance is due to the interference in the extensive hydrogen bonding between the SEs monomers, which will be further discussed later.

4.4.4 Molecular organization/diffraction properties of oleogels

The effect of adding SFL on the self-assembly of SEs was investigated with X-ray diffraction. A similar approach was used to elucidate the modification in self-assembly of oleogels structured with LMOGs (Bodennec et al., 2016; Nikiforidis & Scholten, 2014; Pernetti, van Malssen, Kalnin, et al., 2007). We observed that the peak position of the SEs mono-component (Figure 4.13a and c) was located between 1° to 2.0° (2θ), which is consistent to the peak reported for sucrose stearate (HLB) (Jandacek & Webb, 1978; Szuts et al., 2007) (Figure 4.13). It has been reported that sucrose polyesters pack similar to α and β' phase of TAGs (Jandacek and Webb, 1978). The phase obtained in sucrose polyester was more stable compared to the analogous phase in TAGs (Herrington & Sahi, 1988; Jandacek & Webb, 1978). Therefore, we suggested that the 10:0 SEs:SFL packed analogous to α- and β'-phase of TAGs as the melting temperature and SAXD peak obtained were identical with studies on sucrose polyesters (Herrington & Sahi, 1988; Jandacek & Webb, 1978; Szuts et al., 2007). Meanwhile, sucrose monoester, diester and triester tend to pack into spherical micelles (Becerra et al., 2008; Chansanroj & Betz, 2010; Kawaguchi et al., 1991; Molinier et al., 2006; Molinier et al., 2007; Sandoval, Ortega, Sanchez, Morales, & Gunther, 2015). On the other handd, Pernetti et al. showed that the SFL alone at 8 wt% in oil did not produce any diffraction (Pernetti, van Malssen, Kalnin, et al., 2007), which agrees with the absence of gel formation in SFL mono-component system.

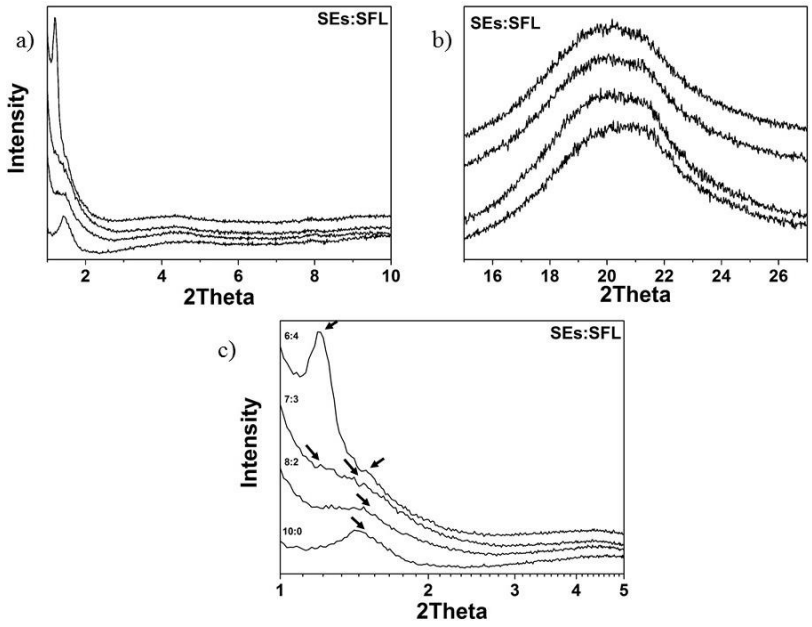

Figure 4.13 SAXD (a and c) and WAXD (b) patterns of oleogels.The ratios depicted on the graph represent the sucrose esters(SEs) to sunflower lecithin(SFL) combination oleogels at 10 %wt total structurant's concentration.

The oleogels of 7:3 and 6:4 SEs:SFL revealed a peak which was absent in SEs mono-component oleogel. This additional peak was located between 1.6° to 1.8° (2θ) (Figure 4.13a and c). Nikiforidis and Scholten suggested that the change in peak's pattern (broadening of peak) can be related to the presence of either a lamellar phase or inverse cylindrical micelles configuration for lecithin-tocopherol combination (Nikiforidis & Scholten, 2014),. The substantial difference of our diffraction to the diffraction of lecithin-tocopherol was the presence of additional peak in the bi-component oleogels that also in agreement with the change in morphology (Figure 4.10, 4.11, and 4.12). Hence, we speculate on the formation of a supramolecular structure consisting mainly of inverse cylindrical micelles (Bodennec et al., 2016; Nikiforidis & Scholten, 2014; Szuts et al., 2010), as suggested for lecithin-tocopherol, lecithin-water, and sucrose ester systems. Although the peak position are difficult to resolve, the exhibited diffraction pattern is sufficient to elucidate the different self assembly. Yet, further investigation is necessary to confirm the supramolecular structure.

4.4.5 Enhanced viscoelastic properties of oleogels as a result of self-assembly modification

It is well known that SEs or SFL by itself cannot structure non-polar solvents (Bodennec et al., 2016; Nikiforidis & Scholten, 2014; Pernetti, van Malssen, Kalnin, et al., 2007). SEs in oil are difficult to disperse which directly limits their potential as a structurant for non-polar solvent (Herrington & Sahi, 1988; Szuts et al., 2007). SFL gels non-polar solvents only when combined with sorbitan-tristearate (Pernetti, van Malssen, Kalnin, et al., 2007), α-tocopherol (Nikiforidis & Scholten, 2014), and water-induced gelation (Bodennec et al., 2016). The gelation of these systems is attributed to the change in self-assembly, similar to our of SEs:SFL oleogels (Figure 4.2). Hashizaki and co-workers, however, dispersed the mixture of lecithin-hydrophilic sucrose ester in methanol/ethanol to obtain a uniform dispersion and to minimize the aggregation of SEs, before adding n-decane oil (Hashizaki et al., 2009). Thus, the most important outcome of this study is the formation of a uniform dispersion and the gelation of SEs without the use of any alcohol/polar solvent (Fanun, Wachtel, et al., 2001; Garti, Aserin, et al., 2000; Garti, Clement, et al., 2000; Hashizaki et al., 2009).

The entropic hydrophobic effect has shown to induce a change in packing geometry of the reverse micelles of lecithin in oil in the presence of tocopherol and also in other amphiphilic-based combinations, leading to crystals/structures formation (Fong et al., 2012; Nikiforidis & Scholten, 2014). Likewise, changes in crystallization and morphology of our bi-component SEs:SFL oleogels can be related to entropic hydrophobic effect. This effect minimizes the hydrophilic head group-solvent interaction, thereby modulating the self-assembly behavior (partitioning effect) of the oleogels (Bodennec et al., 2016; Nikiforidis & Scholten, 2014; Pernetti, van Malssen, Kalnin, et al., 2007). The change in self-assembly behavior is clearly manifested in the SAXD pattern of bi-components against their mono-component (SEs) oleogel. Ultimately, the formation of structure in the bi-component oleogels is the combined effect of entropic hydrophobic effect (Nikiforidis & Scholten, 2014; Pernetti, van Malssen, Kalnin, et al., 2007) and lecithin-induced conformational changes (Hashizaki et al., 2009; Kwon & Kim, 2001; Szuts et al., 2010). Yet molecular characterization on the self-assembly is necessary.

The SEs used in this study are combinations of different sucrose ester monomers that assemble into different structures (organization) in sunflower oil. The sucrose ester monomers with large hydrophilic head are likely to link via hydrogen bonds to avoid contact with sunflower oil (Clemente et al., 2012; Nikiforidis & Scholten, 2014; C. Wang et al., 2012). In the presence of SFL, interference in the hydrogen bonding (between SEs monomers) affects the spontaneous curvature in SEs, which is ultimate for molecular organization (Hashizaki et al., 2009). This is due to the fact that the change in molecular organization parameter can be induced by increasing the effective lipophilic volume of surfactant (Rodriguez-Abreu et al., 2005) and/or through inter-molecules interaction, hydrogen bonding (Fong et al., 2012). Stubenrauch explained how molecular architecture and interaction with additive molecules (co-surfactant) influence the behavior of self-assembled host surfactants (Stubenrauch, 2001). The interaction between host and additive surfactant found to decrease the molecular bending (Bodennec et al., 2016; Hashizaki et al., 2009; Shchipunov et al., 1999), thus hinders the formation of aggregates (globular structure) which cause phase separation (Stubenrauch, 2001). Therefore, in the presence of SFL, instead of hydrogen bonding among the host monomers, a possible linkage to the hydrophilic part of SFL is plausible.

4.5 Conclusions

Based on the growing interest in oleogelation by different structurants (Bin Sintang et al., 2017; Bodennec et al., 2016; Bot & Agterof, 2006; Doan et al., 2015; Nikiforidis & Scholten, 2014; Patel, Schatteman, De Vos, et al., 2013; Pernetti, van Malssen, Kalnin, et al., 2007; Rogers et al., 2008), which are used as an alternative to TAGs based structurant, this work has demonstrated the capability of using surfactants as a structuring building block to structure sunflower oil. Sunflower oil was structured by a combination of SEs and SFL, while no gelation was observed for the SEs and SFL mono-component systems. Additionally, we have shown for the first time the gelation of SEs and SFL even occurred at concentrations lower than for the reported lecithin-sorbitan tristearate (Pernetti, van Malssen, Kalnin, et al., 2007) and lecithin-tocopherol (Nikiforidis & Scholten, 2014) oleogels. The presence of SFL in the system helps to control the colloidal aggregation of SEs, as a result of the extensive hydrogen bonding between the SEs and SFL monomers. Hence, this approach capitalized the colloidal concept in modulating the aggregation and self-assembly, producing a better structure.

Contrary to the approach reported by Hashizaki and co-workers (Hashizaki et al., 2009) who used ethanol solvent to obtain uniform dispersion of SEs and lecithin in n-decane oil, and in conventional preparation of microemulsions (Fanun, Wachtel, et al., 2001; Garti, Aserin, et al., 2000; Garti, Clement, et al., 2000), we applied the melt-mixing technique to obtain a uniform dispersion of SEs in oil. The use of ethanol or any other organic solvent in other preparations, restricts the applicability of those systems in food.

Generally, the interaction modifies interfacial behavior in terms of packing configuration and curvature of host surfactant (Shchipunov et al., 1999; Stubenrauch, 2001). Consequently, host-surfactant (SEs) in a bi-component system adopts a different packing configuration than in its mono-component. The modulation in self-assembly can be observed in the increase of |G*| and alteration in the crystallization and melting profiles. In SAXD analysis, as SFL were added into the SEs-sunflower oil system, the SAXD pattern changed which signified modification in self-assembly (Nikiforidis & Scholten, 2014; Pernetti, van Malssen, Kalnin, et al., 2007). Likewise, changes were also recorded in the morphology of oleogels visualized using optical, electron, and confocal microscopes.

4.6 Perspectives

Further research could be dedicated to a more detailed understanding on the phase behavior and structural formation of SEs:SFL in sunflower oil using synchrotron technique (Chapter 5). Additionally, our study has shown gelation of sunflower oil by using commercial SEs, which consist of heterogeneous monomers. Thus, it is of great interest for the colloidal chemist to study in details the dynamics of self-assembly of this commercial SEs and the combination with SFL in edible oil.

Chapter 5 Elucidating the molecular organization of oleogels using synchrotron radiation

5 Elucidating the molecular organization of oleogels using synchrotron radiation

5.1 Research strategy/hypothesis

True insight in the macroscopic behavior of oleogels can only be achieved by understanding the nano-scale structure formation of the structurants in the liquid oil (Figure 1.2) (Marangoni et al., 2012; Yu, Lin, Yu, & Liu, 2015). In this chapter, synchrotron radiation X-ray diffraction was used to elucidate the synergistic interactions of the oleogelator combinations studied in Chapters 2, 3, and 4. For each oleogelator combination, a ratio best demonstrating the synergism was chosen. This chapter gives an overview of the conclusions drawn in each chapter. The use of the synchrotron radiation provides further insights in the microstructure and interactions between structurants, which serve as the basis to explain the enhanced rheological behavior of bi-component oleogels compared to their respective mono-component.

5.2 Introduction

An oleogel consisting of both crystal networks and liquid oil, has both liquid-like properties (viscous) and solid-like properties (elastic) (Chen et al., 2010; Stokes & Frith, 2008; Weiss, 2014). A soft material, such as an oleogel, consists of a collection of clustered structures or flocs at microscale held together by non-covalent intermolecular interactions to form a three-dimensional network (Co & Marangoni, 2012; Terech & Weiss, 1997). These interactions, combined with the properties at different structural levels (i.e nanoscale: packing parameter (polymorphic type); mesoscale: the size and shape of primary crystals), control the mechanical properties of soft materials (Marangoni et al., 2012; Weiss, 2014). The structural formation begins with the assembly of molecules into primary crystals, as the temperature decreases below the transition temperature. The primary crystals then grow into a network, consisting of a collection of crystals flocs/domains (Marangoni et al., 2012; Yu et al., 2015).

Several attempts have been initiated to correlate the influence of different length-scales in gels to their macroscopic properties. The group of Marangoni has studied the factors that influence the rheological performance of fat-based gels (i.e., margarines

and shortenings). The results showed that the rheological performance of a gel is a combined effect of solid content, crystals' size and morphology, thermal behavior and polymorphic form (Acevedo & Marangoni, 2015; Litwinenko et al., 2002; Marangoni & Pink, 2015; Marangoni & Rousseau, 1996; Narine & Marangoni, 1999). Later, it was found that mainly the fractal dimension of fat crystals in a gel, and not the solid fat content, influences the rheological performance (Tang & Marangoni, 2006a, 2006b).

The spatial distribution or fractal dimension of a network relies on the crystallization process, which determines the properties of the primary crystals (nanoplatelets). The nanoplatelets are the structuring units of crystal flocs (Marangoni et al., 2012; Marangoni & Pink, 2015). Characterization of nanoplatelets is now possible due to the advances in analytical techniques, such as electron microscopy and X-ray diffraction (ultra-SAXS: the scanning range goes below 1° (2θ), which is the lowest limit of conventional XRD) (Acevedo & Marangoni, 2010; Acevedo et al., 2011; Peyronel, Pink, et al., 2014; Peyronel, Quinn, et al., 2014; Ramel et al., 2016). The microstructures observed under microscope are formed through hierarchal events, involving different length-scales (Acevedo & Marangoni, 2015; Ramel et al., 2016). All these events are traced back to the initial assembly/crystallization of molecules at nanoscale, which is the main precursor for the macroscale structure (Acevedo & Marangoni, 2015; Marangoni et al., 2012).

The crystallization process is investigated using differential scanning calorimetry, microscopy, and x-ray diffraction (Nikiforidis, 2015). X-ray diffraction has shown to be a practical instrument to study the molecular arrangement of a structurant in oil, and has been used in many studies (Blach et al., 2016; Bot & Flöter, 2011; Lopez-Martinez et al., 2015; Marangoni et al., 2012; Tavernier, Doan, et al., 2017). However, the power of a lab-scale X-ray source is often insufficient to investigate the molecular arrangement of oleogelators in liquid oil. Only a synchrotron radiation source, generated by a beam of particles, is capable to overcome this limitation (Cheng et al., 2017).

The application of synchrotron X-ray diffraction has been used extensively in fat crystallization and soft-matter assembly. In fat crystallization, it was used to postulate the role of seed crystals in the crystallization of palm oil (Verstringe et al., 2014). In interfacial crystallization, the technique helped to elucidate the arrangement of fat

crystals at the interface (Wassell et al., 2012) and the effect of tempering on the partial coalescence of fat (Moens et al., 2015).

In this chapter, synchrotron X-ray diffraction (SAXD and WAXD) was employed to investigate the crystallization/assembly process. At a cooling rate 10°C/min and a 10-minute holding time at 5°C, the molecular arrangement of the structurants was investigated. The results of the different oleogels were compared to obtain a thorough understanding on the influence of different structures. The understanding is beneficial to elucidate important parameters such as the type packing and polymorphic forms on the structuring capability. Thus, ease researcher in exploring potential structurants in the future, and advancing the field of edible oil structuring.

5.3 Materials and methods

5.3.1 Materials

The materials used in this chapter were explained in their respective chapters (Chapter 2, 3, and 4).

5.3.2 Oleogels preparation

The same sample preparation was done as in the respective chapters and sections. The selected oleogels are summarized in Table 5.1.

Table 5.1 The list of selected oleogels for synchrotron analysis

Oleogels	Composition
Fat capsules based oleogels (Chapter 2)	
Fully hydrogenated rapeseed oil	12 wt% FHRO
Methylcellulose coated crystals	12 wt% Fat capsules
Monoglycerides and phytosterols combinations (Chapter 3)	
Distilled monoglycerides (DM)	10 wt% and 6 wt% DM
Phytosterols (PSs)	10 wt% PSs
Bi-component	10 wt% 6:4 (DM:PSs)
Sucrose esters and lecithin combinations (Chapter 4)	
Sucrose esters (SEs)	10 wt% SEs
Bi-components	10 wt% 9:1 and 6:4 (SEs:SFL)

5.3.3 Time-resolved synchrotron X-ray diffraction

The molecular organization of the selected oleogels was investigated using synchrotron XRD. Both SAXD and WAXD experiments were performed on the Dutch-

Belgian (DUBBLE) beamline BM26B at the European Synchrotron Radiation Facility (ESRF) in Grenoble (France). The experiment was conducted at a fixed wavelength (λ) of 1.33 Å. A linear 300K photon counting 2D Pilatus detector was used for WAXD, whereas a large aread high sensitive photon counting 2D Pilatus detector was used to collect the SAXD images.

The oleogels were enclosed in glass capillaries. The temperature of the stage was controlled by a Linkam hot stage. All the oleogels were subjected to the following time-temperature profile: holding at 90°C for 10 min, cooling at 10°C/min to 5°C, holding at 5°C for 10 min, and heating at 5°C/min to 90°C.

Diffraction patterns were taken every 2°C during the cooling and heating step. Known reflections of standard silver-behenate and empty glass capillary were used for calibration. The patterns are presented as a function of q-range (1/nm). All diffraction patterns were corrected for the detector response, normalized to the intensity of the primary beam and corrected by the sample absorption before performing the background subtraction.

5.4 Result and discussion

5.4.1 The influence of methylcellulose on the formation of a composite oleogel with fully hydrogenated rapeseed oil

5.4.1.1 The effect of a colloidal polymer on the crystallization of fully hydrogenated rapeseed oil

The incorporation of hydrophilic polymers as structurants into sunflower oil has been discussed, in term of physical, thermal, polymorphic, and microstructural properties (Chapter 2). Previously, the thermal and polymorphic properties of the resulting oleogels remained unaffected compared to the reference containing solely FHRO, as described in Section 2.4.2.1.1. However the molecular organization of FHRO in the presence and absence of polymer during cooling remains vague. As been thoroughly discussed in literature, the presence of impurities or minor components have an influence on the crystallization behavior of fats (Bayes-Garcia et al., 2015; Patel & Dewettinck, 2015; Sato et al., 2013; Smith et al., 2011).

Upon cooling, FHRO oleogel, the reference, crystallized into an α-polymorph, indicated by the appearance of peaks at 57.12Å (SAXD) and 4.12Å (WAXD), as shown respectively in Figure 5.1a and 5.2a. The diffraction pattern of SAXD exhibited the characteristic of lamellar with 2L (stacked into two-fatty acid length) packing observed in all the oleogels of FHRO and capsule-based. The 2L packing characteristic was deduced from the order of the peaks. The peak at 57.12Å was well corresponded to the second (28.56Å) and third (19.04Å) order. In case of 3L, the first-order peak is appeared at low angle (Mykhaylyk & Hamley, 2004), which in that case the 57.12Å now becomes the second-order (Figure 5.1). However, the peak of 28.56Å (third-order) and 19.04Å (fourth-order) do not yield the same value for the first-order peak (Mykhaylyk & Hamley, 2004). Therefore, the corresponding peak order obtained in our study consistently displays 2L layer. Likewise, the oleogel containing MC exhibited a similar diffraction pattern in SAXD and WAXD (Figure 5.1b and 5.2b).

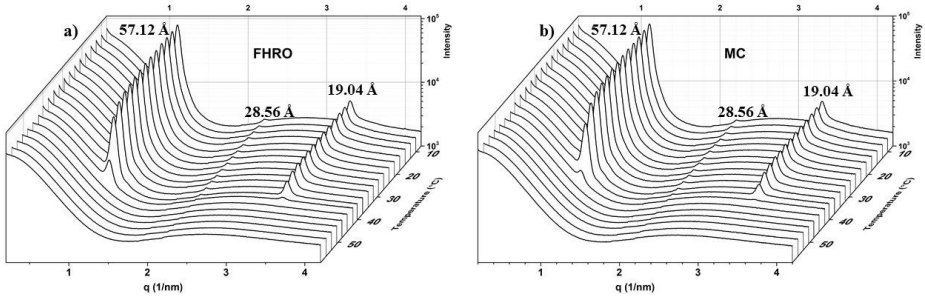

Figure 5.1 SAXD patterns of oleogels prepared from Fully hydrogenated rapeseed oil (FHRO) (a) and fat crystals coated with methylcellulose (MC) (b) during cooling with cooling rate of 10°C/min.

There is no indication in the diffraction patterns that the MC networks has an influence on the crystallization of FHRO. For instance, peaks in SAXD and WAXD profiles appeared at the same temperature in both oleogels.

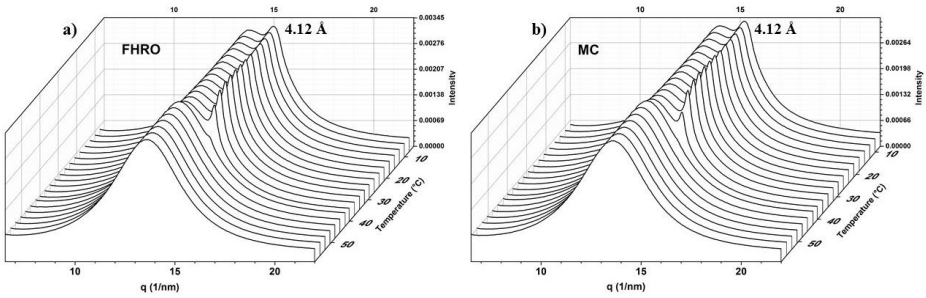

Figure 5.2 WAXD patterns of oleogels prepared from Fully hydrogenated rapeseed oil (FHRO) (a) and fat crystals coated with methylcellulose (MC) (b) during cooling with cooling rate of 10°C/min.

5.4.1.2 The effect of a colloidal polymer on the melting of fully hydrogenated rapeseed oil

During melting, a polymorphic transition was observed. As the temperature increased, the FHRO transitioned from the α-polymorph to the β-polymorph (above 35°C), through solid-mediated transition. This transition can be observed in Figures 5.3 and 5.4 by the decrease in layer thickness (SAXD) to 51.08Å. Additionally, the peak at 4.13Å became smaller along with the appearance of a new peak at 4.56Å. In Section 2.4.2.1.1, the melting profile of the fresh oleogels exhibited a broad exothermic peak prior to the main melting peak. This was indicative of the formation of a β-polymorph, as was observed in this section. The formation of peak at 3.88Å, 3.75Å, and 3.65Å signifies the formation of β' polymorph. Generally, a similar transition from α to β-polymorph was already described for saturated triglycerides during melting (Lavigne, Bourgaux, & Ollivon, 1993). The decrease in layer thickness during transition to β-polymorph is due to the tight packing of acyl tails and tilt to a certain degree, compressing the layer (Chen & Terentjev, 2009; Himawan et al., 2006).

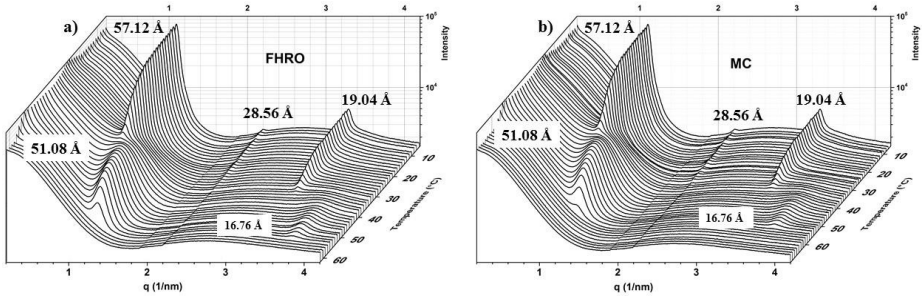

Figure 5.3 SAXD patterns of oleogels prepared from Fully-hydrogenated rapeseed oil (FHRO) (a) and fat crystals coated with methylcellulose (MC) (b) during melting with melting rate of 5°C/min. All the oleogels were held at 5°C for 10 minutes prior to melting.

In the presence of MC, there were no observable changes observed in the diffraction patterns (Figure 5.3b and 5.4b). Hence, support the observation in section 2.4.2.2 and diffraction patterns during cooling. Thus, this is in agreement with the proposed composite oleogel by Chauhan and co-workers (2017a and 2017b), in which the addition of silica particles to induce jamming effect did not influence the crystallization of fat.

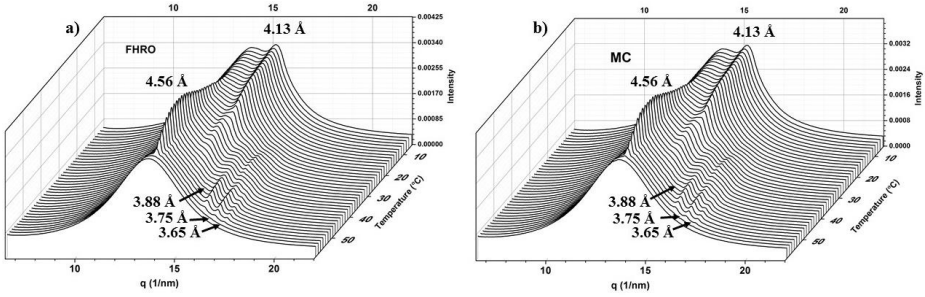

Figure 5.4 WAXD patterns of oleogels prepared from Fully-hydrogenated rapeseed oil (FHRO) (a) and fat crystals coated with methylcellulose (MC) (b) during melting with melting rate of 5°C/min. All the oleogels were held at 5°C for 10 minutes prior to melting.

It has been described and discussed in Chapter 2, the FHRO in oleogels prepared from fat capsules crystallized independently. Likewise, the results from synchrotron X-ray diffraction proof the crystallization and Powder-XRD results discussed in Chapter 2. Furthermore, the β-form and β'-form observed agree with the result obtained in the literature (Danthine & Deroanne, 2003; Deman et al., 1989).

5.4.2 Complexation of phytosterols with monoglycerides and the effect on crystallization

5.4.2.1 The effect of phytosterols on the crystallization of monoglycerides

Complexation of sterols with phospholipids in the phospholipid membrane has shown to influence the physical properties of the membrane, due to the condensing effect (chain ordering). This leads to a decrease in the permeability of the phospholipid membrane as sterols align themselves between the acyl tails of the phospholipids (Cao et al., 2003). Only a few research has focused on the effect of sterols on monoglycerides in a monoglyceride-based membrane (Crilly & Earnshaw, 1983; Larsson et al., 1978; Michalak et al., 2013). However, similar effects have been found in the monoglyceride-membrane containing cholesterol, in which the permeability of water was reduced with the addition of cholesterol (Crilly & Earnshaw, 1983; Michalak et al., 2013). The enhanced rheological properties of monoglycerides oleogels combined with phytosterols supports this hypothesis (Chapter 3).

As a reference, the crystallization of the mono-component oleogels (DM and PSs) were first analyzed (Figure 5.5 and 5.6). Crystallization or self-assembly of MGs has been extensively studied by means of X-ray diffraction. Chen and co-workers elucidated the formation and transition in the phases of MGs during cooling (Chen & Terentjev, 2009). Figure 5.5a and 5.6a show the diffraction peaks of MGs mono-component at 10 wt% in sunflower oil. During cooling, the sharp peak at 46.89Å, characteristic for an inverse lamellar phase, appeared at 36°C and below. In WAXD, the peak at 4.17Å corresponds to the closest distance of glycerol groups in plane in hexagonal packing, which also denotes the α-polymorph. Whereas, the second twin peak at 4.11Å corresponds to the distance between the neighboring polar heads in two planes of the bilayer (Chen & Terentjev, 2009).

Several reports deal with the crystalline phases of PSs, especially β-sitosterol (Bot & Flöter, 2011; Christiansen et al., 2002; Craven, 1976; Moreno-Calvo et al., 2014; Ribeiro et al., 2016; Rossi, Sacanna, & Velikov, 2011; Vaikousi et al., 2007; von Bonsdorff-Nikander et al., 2003; Zychowski et al., 2016). The crystallization of PSs at 10 wt% in sunflower oil exhibited two intense peaks in SAXD, indicating pseudobilayer arrangement of PSs molecules (Bot & Flöter, 2011; Rossi et al., 2011). The measured

bilayer thickness was 37.18Å (Figure 5.5b), which is close to the value (36Å) obtained by Rossi and co-workers (Rossi et al., 2011). In the WAXD region, PSs produced three peaks simultaneously at the beginning of crystallization (Figure 5.6b). The peak at 5.84Å is the packing distance of the sterol ring. The peaks at 4.69Å and 4.74Å indicative of the type of crystals, and the peak at 4.55Å corresponds to the packing of alkyl tail in triclinic configuration (Craven, 1976; Moreau et al., 2002; Moreno-Calvo et al., 2014; Ribeiro et al., 2016; Rossi et al., 2011; Rossi et al., 2010; Vaikousi et al., 2007; Zychowski et al., 2016).

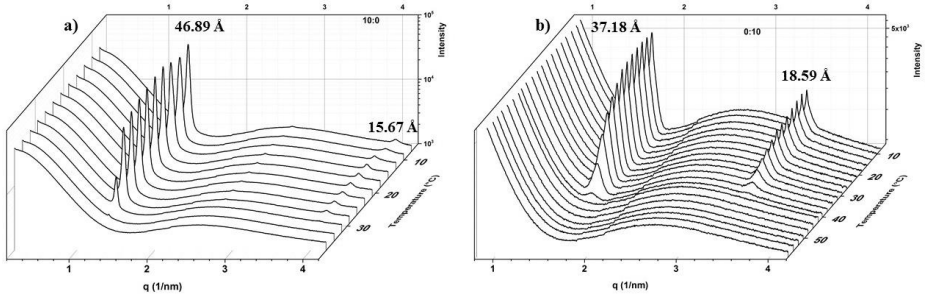

Figure 5.5 SAXD patterns of 10 wt% oleogels prepared from distilled monoglycerides (DM) (a) and phytosterols (PSs) (b) during cooling with cooling rate of 10°C/min.

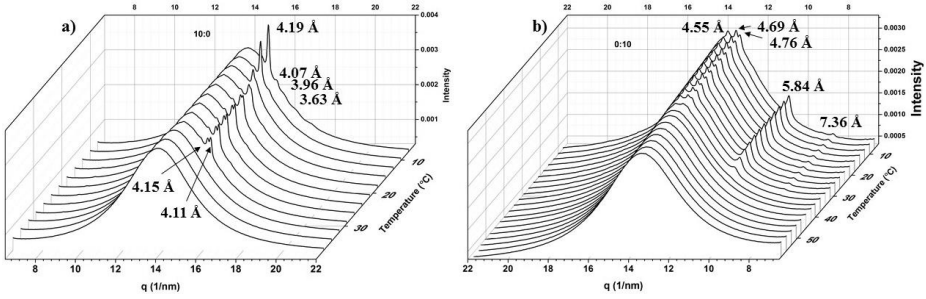

Figure 5.6 WAXD patterns of 10 wt% oleogels prepared from distilled monoglycerides (DM) (a) and phytosterols (PSs) (b) during cooling with cooling rate of 10°C/min.

To understand the effect of adding PSs to the DM oleogel, we selected oleogels containing 6:0 and 6:4 DM:PSs (Figure 5.7 and 5.8). The former serves as a reference to compare the kinetics of crystallization at reduced concentration. Similar to the 10:0,

the 6:0 DM:PSs exhibited peaks at approximately the same position in both SAXD and WAXD (Figure 5.7a and 5.8a).

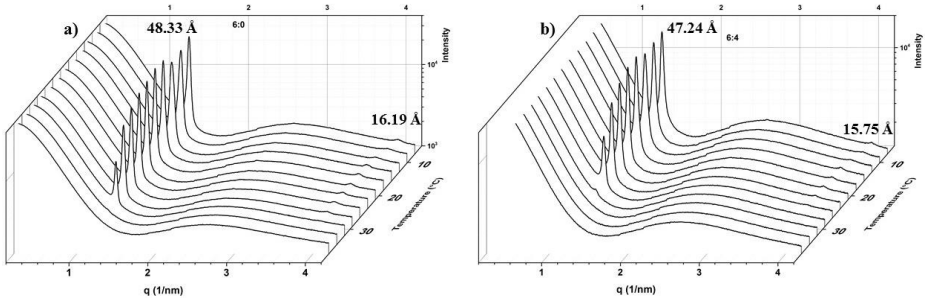

Figure 5.7 SAXD patterns of oleogels prepared from distilled monoglycerides (DM) at 6 wt% (6:0) (a) and the combination of DM and phytosterols (PSs) at 6:4 (10 wt% total structurant) (b) during cooling with cooling rate of 10°C/min.

At the same concentration of DM, adding PSs affected the kinetics of the inverse lamella formation of DM. This effect was manifested in the delay of appearance of the first SAXD peak (Figure 5.7a and 5.7b). The putative complexation of PSs and DM is the result of hydrogen bonding (Gater et al., 2013), which affects the inverse lamellar formation of DM (Chen & Terentjev, 2009). It is worth noting that no corresponding peaks of PSs appear during cooling of the bi-component oleogel (Figure 5.7b and 5.8b). This could be due to low concentration of PSs (4 wt%) or the effect of DM on the crystallization of PSs. Stabilizers or emulsifiers have shown to affect the crystallization of PSs (Christiansen et al., 2002; Engel & Schubert, 2005; Rossi et al., 2011; von Bonsdorff-Nikander et al., 2003).

The incorporation of PSs also affected the corresponding DM peaks in the WAXD region. The characteristic twin-peak of 4.17Å and 4.11Å in the WAXD was explained in the 10 wt% DM mono-component (Chen & Terentjev, 2009). Interestingly, the twin-peak merged into a single peak in the bi-component oleogel (Figure 5.8b), which indicates that the complexation between PSs and DM affects the packing of the DM. A similar result was also observed in the cholesterol incorporated phospholipid membrane (Bach & Wachtel, 2003; Shaikh et al., 2006; Smith et al., 2012).

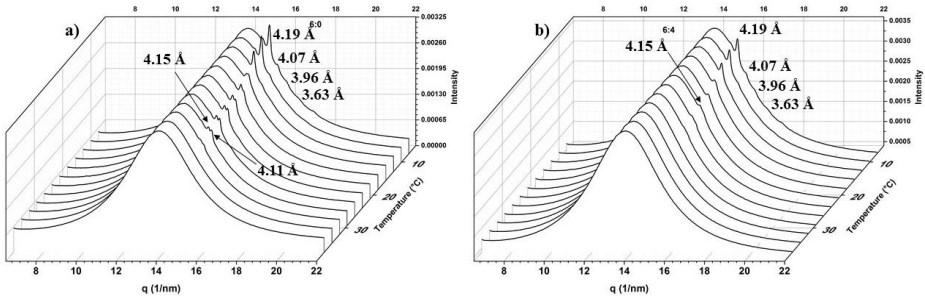

Figure 5.8 WAXD patterns of oleogels prepared from distilled monoglycerides (DM) at 6 wt% (6:0) (a) and the combination of DM and phytosterols (PSs) at 6:4 (10 wt% total structurant) (b) during cooling with cooling rate of 10°C/min.

5.4.2.2 The effect of phytosterols on the melting of monoglycerides

All the oleogels were subjected to isothermal crystallization at 5°C for 10 minutes prior to melting. The diffraction pattern during melting for both DM and PSs mono-component at 10 wt% in sunflower oil are presented in Figure 5.9 and 5.10. The DM mono-component oleogel exhibited a transition in the main peak of SAXD at temperature between 40°C and 50°C. The bilayer thickness changed from 46.89Å to 46.54Å at higher temperature (Figure 5.9a). Additionally, a new peak at 4.55Å appeared along with the disappearance of the peak at 4.18Å in WAXD region at approximately 40°C and above, characteristic of an α- to β-polymorph transition (Figure 5.9a).

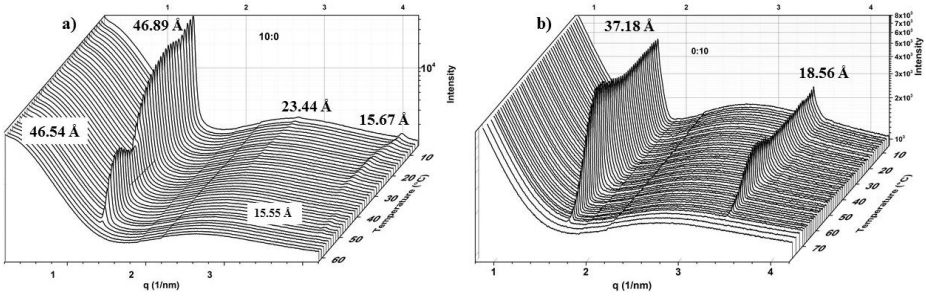

Figure 5.9 SAXD patterns of 10 wt% oleogels prepared from distilled monoglycerides (DM) (a) and phytosterols (PSs) (b) during melting with heating rate of 5°C/min. The oleogels were held at 5°C for 10 minutes prior to melting.

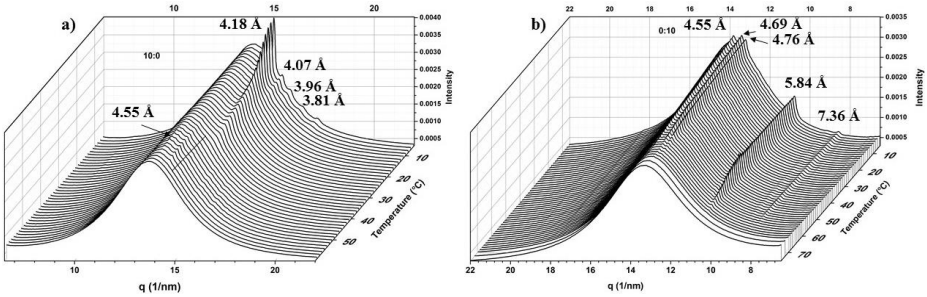

Figure 5.10 WAXD patterns of 10 wt% oleogels prepared from distilled monoglycerides (DM) (a) and phytosterols (PSs) (b) during melting with heating rate of 5°C/min. The oleogels were held at 5°C for 10 minutes prior to melting.

In the β-polymorph, the alkyl tail of DM is tilted to a certain angle which decreases the lamellar thickness due to a tighter packing (Chen & Terentjev, 2009). In the PSs oleogel, there was no noticeable transition during melting (Figure 5.9b and 5.10b). All the peaks remained at the same position without any formation of a new peak.

The 6:0 DM:PSs produced the same pattern as in the 10:0 oleogel, at a slightly different temperature (Figure 5.11). The 6:0 DM:PS exhibited indistinguishable transition peak to β-polymorph in WAXD region (Figure 5.12a). Still, clear differences were observed between 6:0 and 6:4 during cooling in SAXD region (Figure 5.11 and 5.12).

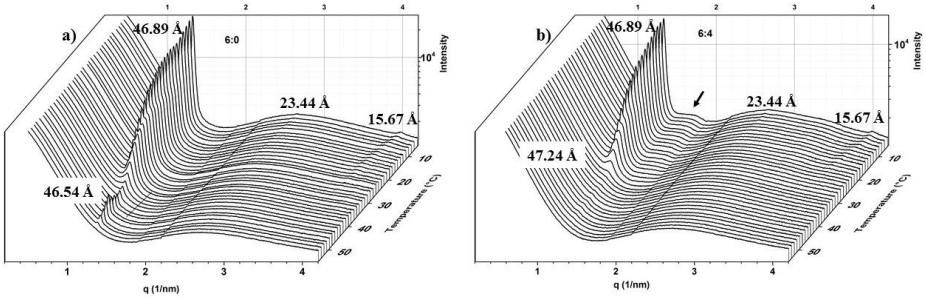

Figure 5.11 SAXD patterns of oleogels prepared from distilled monoglycerides (DM) at 6 wt% (6:0) (a) and the combination of DM and phytosterols (PSs) at 6:4 (10 wt% total structurant) (b) during melting with heating rate of 10°C/min. The oleogels were held at 5°C for 10 minutes prior to melting.

Da Pieve and co-workers reported that concentration did not affect the packing of monoglycerides at stable state, thus supporting our finding (Da Pieve et al., 2011). Althought the 6:0 exhibited thicker bilayer than the 10:0 during cooling, this is predominantly due to the different in tilt angle. Nevertheless, the 10:0 and 6:0 DM:PSs oleogels exhibited similar thickness at the start of melting.

The 6:4 bi-component oleogel exhibited additional shoulder-like peak close to the main peak of SAXD (Figure 5.11b). The appearance of that additional peak corresponds to the presence of PSs crystals, as crystallite PSs appear in the same region. PSs appeared to crystallize during the isothermal period because the solubility limit is exceeded as the result of supersaturation. Vaikousi and co-workers reported that the solubility of PSs at room temperature in corn oil is 2 wt% (Vaikousi et al., 2007). Furthermore, the bi-component oleogel exhibited faster melting compared to the corresponding 6:0 oleogel, suggesting an influence of PSs on the crystallization of MGs (DM). This results agree with the observed thermal behavior discussed in Chapter 3.

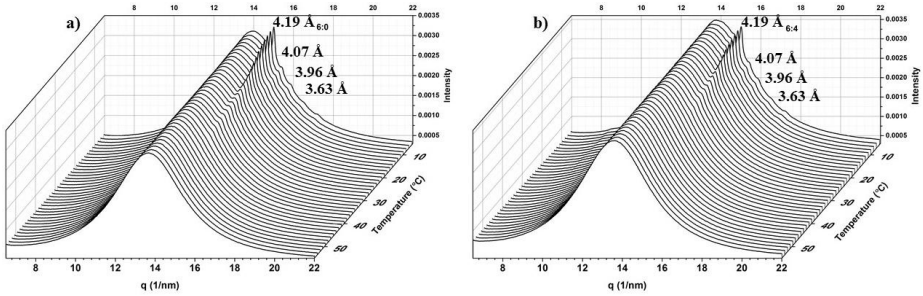

Figure 5.12 WAXD patterns of oleogels prepared from distilled monoglycerides (DM) at 6 wt% (6:0) (a) and the combination of DM and phytosterols (PSs) at 6:4 (10 wt% total structurant) (b) during melting with heating rate of 10°C/min. The oleogels were held at 5°C for 10 minutes prior to melting.

We also observed changes in the diffraction peaks (no observable peak at 4.55Å) of PSs in the bi-component oleogel, which indicate a reduced crystallinity. A similar effect was observed when crystallizing PSs in the presence of emulsifiers (Rossi et al., 2011; Rossi et al., 2010; vons Bonsdorff-Nikander, Lievonen, Christiansen, Rantanen, & Yliruusi, 2003). It is important to mention that the modulation in the crystal properties

allows incorporation of PSs in solubilize form in real food systems. It has been shown in this chapter and supported by the results in Chapter 3 that PSs in combination with DM melt at temperature lower than the melting temperature of neat form. Ultimately, PSs render the melting and crystallization temperature of the DM:PSs bi-component oleogel.

5.4.3 Lecithin-induced transition in the self-assembly of sucrose esters

5.4.3.1 Dynamics of self-assembly modification during cooling

As described in Chapter 4, mixing SEs with SFL modulated the self-assembly of the SEs and led to gelation of bi-component oleogels. The modulation of the self-assembly of SEs was correlated to the hydrogen bonding established between SEs and SFL, which influenced the curvature angle of SEs (Clemente et al., 2012; Fong et al., 2012; Leser et al., 2006; C. Wang et al., 2012). To explain the assembly of SEs in sunflower oil, it is important to stress that SEs contain heterogeneous monomers of different degree of substitution (regioisomers). Roughly, the SEs used, are comprised of sucrose polyester (±58%) and low-esterified sucrose (±42%) (mono-, di-, and tri-ester), which are commercially used in food applications. Sucrose polyester and low-esterified sucrose exhibit different molecular packings in a particular solvent (Becerra et al., 2008; Fanun, Wachtel, et al., 2001; Herrington & Sahi, 1988; Jandacek & Webb, 1978; Molinier et al., 2006; Molinier et al., 2007).

Upon cooling, the SEs mono-component oleogel exhibited five diffraction peaks at the transition temperature (Figure 5.13a). It has been reported that sucrose polyester assembles into lamella, where the packing is analogous to the α- and β' phases of TAGs. However, the packing of sucrose polyester is stable and does not undergo polymorphic transition over time, in contrast to TAG crystals (Herrington & Sahi, 1988; Jandacek & Webb, 1978). Low-esterified sucrose packs into micelles when dispersed at low concentration in a solvent (Fanun, Wachtel, et al., 2001; Kawaguchi et al., 1991; Molinier et al., 2006; Molinier et al., 2007; Rodriguez-Abreu et al., 2005; Sandoval et al., 2015).

Based on the diffraction results, SEs could be structured in two different ways. Firstly, the SEs mono-component oleogel could be packed into lamellar and micellar structures. The characteristic order of peaks, first (57.12Å), second (28.56Å), and third

(19.04Å) peaks is in lamellar order (Herrington & Sahi, 1988; Jandacek & Webb, 1978). The remaining two peaks at 26.29Å and 17.55Å could be attributed to the formation of micelles (Cardiel et al., 2015). Secondly, the SEs could be packed into a bilayer cylindrical hexagonal structure, which is characterized by a twin-peak of the second order (28.56 and 26.29Å) (Yaghmur, de Campo, Sagalowicz, Leser, & Glatter, 2005). However, bilayer cylindrical hexagonal structure is not thoroughly reported in literature which renders a precise determination of structure difficult (Hyde, 2001).

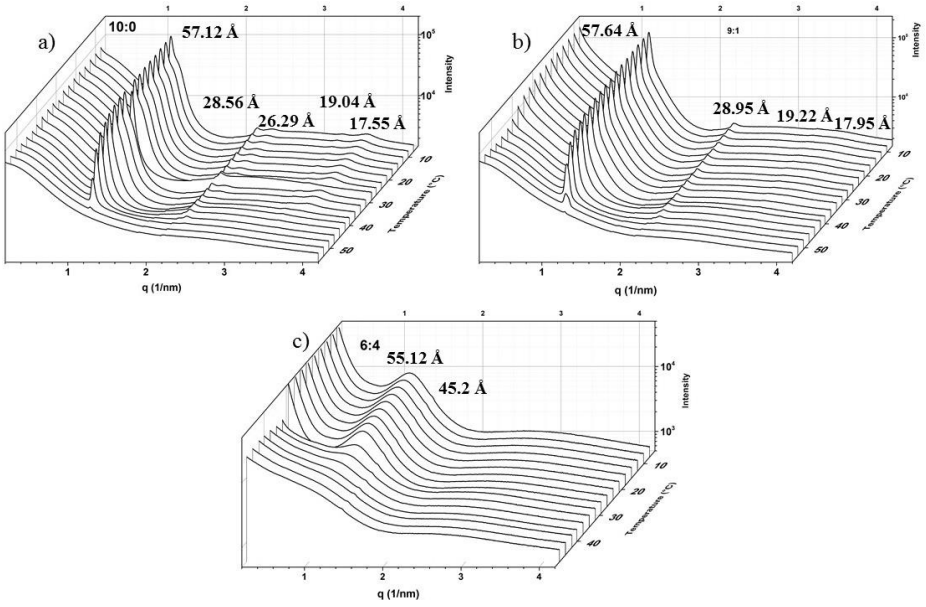

Figure 5.13 SAXD patterns of oleogels prepared from the combination of Sucrose esters (SEs):Lecithin (SFL) at 10:0 (a), 9:1 (b), and 6:4 (c) ratios in sunflower oil at total structurant of 10 wt%. The diffraction pattern was obtained during cooling at cooling rate of 10°C/min.

In a bi-component system, the addition of SFL changed the pattern of the diffraction peaks. The 9:1 SEs:SFL oleogel exhibited some similarity with the diffraction patterns of the SEs mono-component oleogel (Figure 5.13b). Differences could be seen in the peak position, which shifted to higher distance (57.64Å), and a decrease in the number of peaks from five to four. It has been reported that the addition of a co-surfactant interferes with the non-covalent hydrogen bonding of the host surfactant, inducing changes in the curvature angle (Garti, Clement, et al., 2000; Hashizaki et al., 2009; Nikiforidis & Scholten, 2014; Shchipunov, 2001). Considering the first hypothesis, SFL

could have interfered with the intermolecular hydrogen bonding of SEs monomers. Consequently, the curvature angle of SEs changes which stabilizes the lamellar structure. The 9:1 SEs:SFL oleogel exhibited the characteristic order of peaks of a lamellar conformation (57.64, 28.95, and 19.22). The SFL particularly affected the low-esterified sucrose, as this is the component with a curvature angle leading to a non-lamellar structure.

Adding more SFL into the oleogel led to a complete transition in the self-assembled structure. The 6:4 SEs:SFL oleogel exhibited a different diffraction pattern than the corresponding 10:0 and 9:1 SEs:SFL oleogels. The characteristic order of peaks did not belong to lamellar, as the second peak (45.2Å) in 6:4 did not correspond to the first order peak (55.12Å). To explain this change, the overall composition of 6:4 bi-component oleogel should be considered. As described previously, SEs comprise of sucrose polyester and low-esterified sucrose, which have different packing in sunflower oil. On the other hand, SFL is reported to form micelles in non-polar solvent, alike the low-esterified sucrose. Proportionally, adding more SFL that has similar behavior as low-esterified sucrose, changes the packing behavior of the overall system to favor a micellar-like structure (non-lamellar).

Comparable to the effect observed in the 9:1 combination, localization of SFL in the palisade layer of SEs affected the spontaneous curvature angle (Cardiel et al., 2015; Hashizaki et al., 2009). This induces the transition from spherical micelles to a cylindrical hexagonal structure. Additionally, the 6:4 SEs:SFL displayed the 45.2 Å peak even above its transition (crystallization) temperature (Section 4.4.1), indicating a liquid crystal mesophase. A similar mesophase transition was observed in sucrose ester-based microemulsion in which co-surfactant (alcohol) was added (Fanun, Wachtel, et al., 2001; Garti, Aserin, et al., 2000; Garti et al., 1999; Rodriguez-Abreu et al., 2005; Stubenrauch, 2001).

5.4.3.2 Dynamics of structures during melting

Overall, the structural features at the beginning of melting (at 5°C) were similar to the structures during cooling in all the oleogels. This indicates that no structural transition occurred during the ten-minute isothermal at 5°C. Figure 4.4 (Chapter 4) shows that the highest offset melting temperature was 73.76°C in 9:1 SEs:SFL. Here, it is interesting to note that the 10:0 and 9:1 SEs:SFL oleogels revealed new peaks at

higher temperature (Figure 5.14 a and b). Comparable as during cooling, the 6:4 exhibited a different diffraction pattern than the 10:0 and 9:1 SEs:SFL oleogels, with an additional peak at 45.2Å. This peak remained present in the entire temperature region, which indicates the formation of mesophase structures in 6:4 SEs:SFL. Similar behavior was observed in studies where SEs of different HLBs were combined with lecithin, in which worm-like or tubular micelles were formed, indicated by the increase in viscosity and diffraction pattern at higher temperature (Engelskirchen et al., 2006; Fanun, Wachtel, et al., 2001; Hashizaki et al., 2009; Ullrich et al., 2008). Yet, detailed analysis is required to elucidate the structural features of SEs:SFL bi-component oleogels.

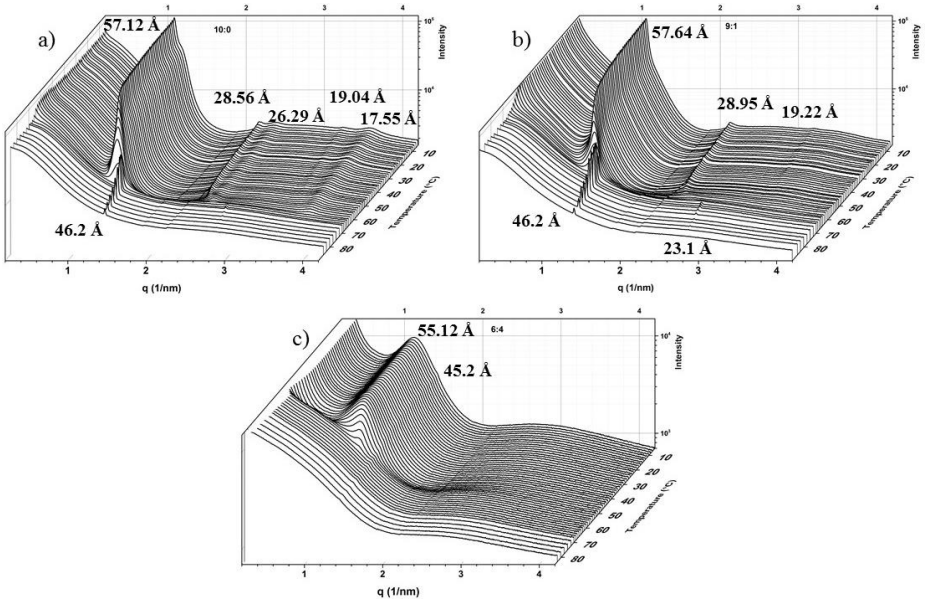

Figure 5.14 SAXD patterns of oleogels prepared from the combination of Sucrose esters (SEs):Lecithin (SFL) at 10:0 (a), 9:1 (b), and 6:4 (c) ratios in sunflower oil at total structurant of 10 wt%. The diffraction pattern was obtained during melting with heating rate of 5°C/min. All the oleogels were kept at 5°C for 10 minutes prior to melting.

5.4.4 Contrasting the structural features of different oleogels with their rheological properties

In general, crystalline particles (nanoplatelet ~100 nm) represent the fundamental unit of a fat crystal network (Ramel et al., 2016). How these crystalline particles aggregate will determine the overall performance of a material measured at the macroscale level (Marangoni et al., 2012; Yu et al., 2015). Environmental factors such as cooling rate and temperature have an influence on the formation of crystalline particles (Acevedo & Marangoni, 2010; Acevedo et al., 2011). As presented in the previous section, there are observable interactions during the crystallization of the bi-component systems, except for the fat capsule-based oleogels. Those effects will influence the macroscopic properties (Marangoni et al., 2012; Yu et al., 2015).

For discussion, we selected different types of oleogels and presented their properties in Table 5.2. It is important to emphasize that this section aims to briefly discuss structure-function relationship of the oleogels based on the higher |G*| values. However, higher modulus is not always necessary in real food systems. Therefore, the term promising rheological properties (higher |G*|) was used to avoid misconception and unfair justification of the oleogels. Additionally, this section serves to compare the different molecular organization to the modulus values.

Table 5.2 The comparison of melting temperature and complex modulus of selected oleogels after three-hour crystallization.

Oleogels	Total structurant concentration (wt %)	Melting temperature (°C)	Complex modulus |G*| (kPa)
DM:PSs oleogels			
10:0	10	54.23±0.39	202.93 ±8.00
6:0	10	54.16±0.53	89.13 ±1.20
6:4	10	51.25±0.05	111.21 ±14.20
Fat capsule-based oleogels			
FHRO(ref)	12	56.63±0.04	10.60 ±0.97
MC	12	56.31±0.14	26.15 ±3.59
SEs:SFL oleogels			
10:0	10	65.82±0.06	0.53 ±0.04
6:4	10	53.56±0.96	29.81 ±0.68

Overall, the synchrotron radiation analysis confirmed the presence of different structural features in the studied oleogels. The oleogels of FHRO-based and DM-

based exhibited lamellar structure, which is common in a conventional structuring system (Acevedo et al., 2011; Chen & Terentjev, 2009; Da Pieve et al., 2011; Deman et al., 1989). It is wise to clarify that the selection of FHRO as one of structurant represents the conventional oil structuring approach which relies on TAGs molecules thus, represents the SFA. Whereas, SEs:SFL bi-components unveiled a SAFiNs-like structure, which is different than the other oleogels. The SEs:SFL spontaneously assembled into a cylindrical shape (tubular-like), growing one-dimensionally and forming a network as the tubules overlapped (Hashizaki et al., 2009; Rodriguez-Abreu et al., 2005).

Although both FHRO-based and MGs-based oleogels exhibited similar packing, DM had higher |G*| than the FHRO (Table 5.2). This difference may arise from the aggregation of crystalline particles to form flocs and thus a network. The network forms as the primary crystalline particles aggregate through non-covalent bonds and van der Waals forces, forming a crystal floc (Acevedo & Marangoni, 2010; Acevedo et al., 2011; Marangoni et al., 2012). This floc then interconnects with other flocs to create space-filling network containing the liquid phase (Marangoni et al., 2012; Ramel et al., 2016).

Another factor to consider, explaining the delineation of the physical performance between FHRO and DM lies in the spatial distribution or fractal dimension of crystalline particles, being the microscale network. The network or fractal-like structure has a big influence on the rheological performance of gels (Lazzari, Nicoud, Jaquet, Lattuada, & Morbidelli, 2016; Marangoni & Rousseau, 1996; Tang & Marangoni, 2006b). Hence, it's clear from this study that the fractality may be the probable explanation, governing |G*| and G 'of FHRO and DM oleogels.

The bi-component 6:4 SEs:SFL oleogel exhibited inverse cylindrical hexagonal structure, which was not observed in the SEs mono-component. This unique structural feature differs from lamella in its assembly and growth dimension. The growth dimension for fibers (in SAFiNs oleogel) is unidimensional with a high aspect (length-to-width) ratio, generally measuring a few tens of nanometers in width and up to several micrometers in length (Buerkle & Rowan, 2012; Vintiloiu & Leroux, 2008; Weiss, 2014). This type of oleogels are therefore comparable to polymer gels. The gels are different in the junction zone, in which SAFiNs oleogels form transient junction (physical cross-

linked) whilst some polymer gels exhibits permanent junction (chemical and physical cross-linked) (Vintiloiu & Leroux, 2008).

The bicomponent 6:4 SEs:SFL oleogel revealed a higher |G*| than FHRO oleogel, but lower than the MGs oleogel. As previously discussed, FHRO differed from the DM due to the difference in fractal dimension. Likewise, the tubules of SEs:SFL grow and overlap forming a mesh-like network. It has been reported that the growth of tubules influences the performance of the gels, by influencing the spatial distribution (branching of tubules). Factors such as cooling rate, impurities, and solvent-gelator interaction influence the crystallization of tubules (Buerkle & Rowan, 2012; Fong et al., 2012; Lan et al., 2015; Rogers et al., 2008). Although this oleogel is sometimes considered as weak, its viscosity in certain cases is higher than gelatin gel due to mesophases (Buerkle & Rowan, 2012; Scartazzini & Luisi, 1988; Shchipunov, 2001; Vintiloiu & Leroux, 2008). In addition, the weak and reversible nature of the non-covalent interactions in SAFiN-based oleogels can result in interesting mechanical properties, such as reversible shear sensitivity and healing (Buerkle & Rowan, 2012). Thus, different parameters should be considered to differentiate the SEs:SFL from the MGs system, which is not within the scope of this study.

Non-covalent intermolecular interactions in gels have influence on the physical performance of the gels (Lupi, Greco, et al., 2016; Wu, Wu, Zou, & Zhang, 2011). FHRO contains TAGs molecules, of which the network is predominantly governed by van der Waals interaction (Acevedo & Marangoni, 2015; Acevedo et al., 2011; Marangoni et al., 2012). On the other hand, MGs, SEs, and SFL are amphiphilic compounds and between which the interaction is mainly governed by hydrogen bonding in combination with either van der Waals interactions, π-stacking, or metal-coordination bonds, depending on the chemical structure (Chen & Terentjev, 2009; Dastidar, 2008; Kato, Mizoshita, & Kishimoto, 2006; Lupi, Greco, et al., 2016; Terech & Weiss, 1997; Vintiloiu & Leroux, 2008; Weiss, 2014). Hydrogen bonding is generally stronger than van der Waals forces among the non-covalent bonding (Hammond & Mezzenga, 2008). Recently, Lupi and co-workers studied the effect of non-covalent intermolecular interaction in monoglyceride (hydrogen bonding) and policosanol (van der Waals) oleogels. However, the policosanol used in their study had a long alkyl tail and hydroxyl group, which provided additional forces and thus formed a harder gel

than monoglyceride (Lupi, Greco, et al., 2016). Still, the gelation speed of MGs was faster than policosanol. Thus, their results demonstrated the influence of intermolecular interaction in governing the physical and gelation properties of oleogels, which can be expended to further characterize our oleogels.

Ultimately, it is the interplay of different physical interactions that lead to the gelling of a matrix (Fong et al., 2012; Vintiloiu & Leroux, 2008). The only constant in the gelation mechanism is the balance required between the solubility and the insolubility of the gelator in a given solvent (Co & Marangoni, 2012; Lan et al., 2015). In other words, to structure sunflower oil, the selected structurants should first solubilize in sunflower oil (gelator-oil interaction), but not completely solubilize, as the formation of building block requires extensive gelator-gelator interactions to self-organize into micro scale structure. The gelator-gelator interaction is pivotal, as it determines the structural building block formed by structurants and the physical properties of the resultant oleogels (Rogers et al., 2008; Yu et al., 2015). Yet, in gelator-gelator interaction, there should also a fine balance between attractive and repulsive forces, as strong attractive force will lead to aggregation thus, agglomeration (Buerkle & Rowan, 2012). Meanwhile, the physical properties at macroscale level is strongly relied on the properties at different length-scales (nano, meso, and micro scale; dependent). In other words, though the resultant polymorph and organization of oleogels are the same, their physical properties are also determined by the network structure (spatial distribution) in case of crystalline based oleogel.

It is interesting to note that similar molecular organization could be observed between FHRO and monoglycerides oleogels, which the latter have better rheological properties. Additionaly, both systems have the same structuring unit which is crystalline particles. This observation signifies that oleogels possess a potential as structuring alternative for TAGs-based structurant. In most of the application studies, the ability to structure edible oil is the main factor in performing application test, which mostly performed randomly. The results from the application tests show that using oleogels as the fat compromise certain quality attributes than the reference, conventional fat. There are few reasons that could be pointed to explain this drawback. Food systems or structures are complex which contain multiple dispersed phases thus, the interactions between ingredients or components are highly probable. This interaction is called matrix effect, for instance interaction of gelatinized starch with fat

in laminated dough (Mattice & Marangoni, 2018). Therefore, it is plausible for oleogelators to lose their functionality during product application. Particularly in the presence of oil-water interface, most of structurants which are amphiphilic lose their functionality as they now conquered with interfacial activity (Floter, 2012). In other applications such as drug delivery and controlled release, however, most of oleogels or organogels are able to produce products with desirable quality and properties. This is because such applications involve a simple system which consists of only solvent, structurants, and functional ingredients (drugs). Therefore, the interaction between structurants and food matrix, and the complexity of food systems are the most probable causes for the failure of oleogels to live up to the standard (de Kruif, 2012; Floter, 2012). To mitigate this shortcoming, comprehensive understanding of oleogels is necessary through in-depth characterization of the structuring behavior and mechanism fundamentally. With that understanding, systematic and scientific approach can be employed to increase success rate in application tests. Nonetheless, with the introduction of more edible oleogels, it provides industries particularly food an abundant choices of structuring alternatives for reformulation and innovation activities prior to discovery of systematic approach for application of oleogels.

5.5 Conclusions

This chapter discussed the effect of an additive on the gelation properties of several structurants. In polymer coated fat crystals, the presence of MC did not influence the crystallization of FHRO, which confirms the results from Chapter 2. During cooling, the correspondent peak of crystallization appeared at the same temperature in FHRO- and fat capsule-based oleogels. In addition, the length of the packing showed the same distance of 57.12Å. Therefore, it can be deduced that MC existed as colloidal particles which strengthen the network of FHRO.

As observed in Chapter 3, the addition of MGs and PSs led to modification in the rheological properties. The improvement in the rheological properties is correlated to the complexation and condensing effect in MGs and PSs mixture, which can be detected with a change in crystallization behavior. The 6:4 combination exhibited a delay in crystallization in comparison to 6:0 MGs:PSs, indicating complexation. The complexation also leads to a condensing effect of PSs, affecting the formation of inverse lamella (SAXD) and the distance between the glycerol head (WAXD), more

particularly the 4.11Å peak. In conclusion, PSs did interfere with the crystal habit of MGs.

Upon combining SEs and SFL, the self-assembly modification induced by SFL on SEs, indicated by the suppression of peaks and formation of new peaks, as observed in the SAXD results. The results clearly show a different diffraction patterns of the 6:4 SEs:SFL oleogel compared to 10:0 SEs:SFL oleogel. The 6:4 SEs:SFL formed reversed cylindrical hexagonal structure, whilst the SEs mono-component exhibited lamellar structure. The complex composition of SEs, containing heterogeneous monomers with different behavior, complicates the structural determination. Additionally, there are limited studies on commercial SEs. Still, the results seem to suggest a change in mesophase.

Related to oil structuring, it can be concluded that different structural features could structure sunflower oil. Although FHRO and MGs produced a similar molecular packing, the latter had higher |G*|. The 6:4 SEs:SFL combination however, produced a different molecular packing than the other structurants. The |G*| were found to be higher compared to FHRO, but lower than MGs. This difference is due to the influence of inter-molecular noncovalent interactions, governing the network and physical properties (Lupi, Greco, et al., 2016; Wu et al., 2011). The interactions are pivotal in the formation of a space-spanning network.

5.6 Perspectives

The study of inter-molecular noncovalent interactions in oleogels provides additional information on the gelation ability of structurants and allows us to understand the gelation process better. It is a new perspective, additional to the current understanding on factors influencing gelation of molecular gels, which are currently limited to the chiral effect and the hydrophobic effect (Co & Marangoni, 2012; Vintiloiu & Leroux, 2008; Weiss, 2014; Wu et al., 2011).

Chapter 6 Current state and future perspectives of oleogelation as an alternative structuring for edible liquid oil

6 Current state and future perspectives of oleogelation as an alternative structuring for edible liquid oil

6.1 General conclusions

Alternative edible oil structuring has gained considerable attention from the researchers and food industry. This is due to the associated risk of a hardstock which contains a high amount of saturated and *trans* fatty acids to non-communicable diseases. Conventionally, hardstock is necessary to provide a structuring building block in the form of crystalline particles to immobilize liquid oil. Additionally, these crystalline particles also impart the organoleptic properties of the end products. Therefore, reformulating lipid-based products with alternative structuring agents (oleogelation) might help to manage the lipid composition (level of saturated and *trans* fatty acids), whilst sustaining the physical and organoleptic properties of end-products. Despite the scientific progress in the field of oleogelation, there is a limited selection of oleogelators that can structure liquid oil. Therefore, this study provides insights in synergistic combinations of two different structurants, to fulfill the need for more edible structurants thus, allow for more innovation in lipid-based products and even in pharmaceutical and cosmetics. Interestingly, most of the structurants found in this study are currently used as food ingredients.

It has been reported that bi-component systems consisting of two different structurants not only can interact but also can improve the structuring ability of their respective mono-component structurants. This is based on specific interactions between the structurants that influence the crystallization or self-assembling of either one of the combined structurants. Nevertheless, it is necessary to find the optimum ratio combination where the improvement can be materialized. This is because excessive addition of modifier might lead to negative effects of the bi-component system. Therefore, this study investigated combined structurants and elucidated the optimum ratio at which promising oil structuring properties, indicated by higher network rigidity (complex modulus |G*|), is materialized. This was clearly manifested in the sucrose esters and lecithin combination oleogels, whereby gel formation was only observed in the combinations and not in the respective mono-components.

Oleogels, either mono-component or bi-component, are categorized into five different categories namely crystalline-based, self-assembly fibrillar networks (SAFiNs), polymer-based, particle-based, and indirect approach. Overall, this study introduces three structurants' combinations, spanning from different oleogels' categories. The fully hydrogenated rapeseed oil – hydrophilic polymers bi-component systems belong to the crystalline-based and indirect approach categories. This is due to the fully hydrogenated rapeseed oil consisting of predominantly triglycerides molecules which crystallize upon cooling. Meanwhile, the incorporation of hydrophilic polymers into the sunflower oil involves an indirect approach due to their different molecular properties (incompatible). Then, the bi-component systems based on monoglycerides – phytosterols belong to the crystalline-based approach, as both structurants show birefringent structures under polarized light microscope. Finally, the sucrose esters – lecithin bi-component systems belong to the self-assembly fibrillary networks, as evidenced from the synchrotron radiation (X-ray).

The synergistic interaction leading to the promising rheological properties, high complex modulus, can be attributed to a specific interaction between the combined structurants. In fact, there is a strong correlation between non-covalent intermolecular interaction(s) and structuring capability of structurants. In this study, however, the intermolecular interactions in the oleogels were not characterized spectroscopically. Nevertheless, the diffraction results obtained from powder X-ray and synchrotron radiation instruments provided qualitative evidence on the contribution of non-covalent intermolecular interactions, especially hydrogen bonding. The contribution of hydrogen bonding on the self-assembly can be detected by the change in the diffraction patterns in SAXD, which is also commonly used to study the self-assembly of microemulsion and other amphiphilic self-assembly systems. Meanwhile, the existence of polymer networks could be ascribed to volume effect, which occupies spaces between crystals.

The selection of fully hydrogenated rapeseed oil, monoglycerides, phytosterols, sucrose esters, and lecithins as the structurants provides general overview of the different in nanoscale assembly and physical properties (complex modulus). The fully hydrogenated rapeseed oil contains predominantly triacylglyceride molecules thus, best representing the current conventional system. Whereas, the majority of the other main structurants are amphiphilic molecules. This study showed that the structuring features of oleogels do not solely rely on lamellar stacking to create a three-

dimensional network, as is the case for conventional structuring through hardstock (TAGs-based). The structuring features can arise from the combined effect of non-covalent interactions and self-assembly processes of amphiphilic molecules. This is supported by the findings from this study that the studied bi-components organize into lamellar and inverse cylindrical hexagonal structures. The formation of these structures was confirmed using powder and synchrotron X-ray diffraction instruments, based on the change in diffraction patterns at small angles. The fully hydrogenated rapeseed oil, monoglycerides, phytosterols, the combinations of monoglycerides and phytosterols, and sucrose esters mono-component system organized into lamellar with predominantly packed into triclinic unit cells (β-polymorph) in sunflower oil. Meanwhile, the combinations of sucrose esters and lecithin in sunflower oil form inverse cylindrical hexagonal structure with no detectable peak at wide angles. Although the information on the different nanostructure is not completely new in oleogelation field, the discovery of the combination of sucrose esters and lecithin is a step forward for SAFiNs-based category.

The formation of the self-assembly structures is also the interplay of non-covalent intermolecular interactions between molecules. Interestingly, hydrogen bonds are capable of inducing changes in the self-assembly orientation, giving rise to different organizations. This is manifested in the bi-component systems comprising of sucrose esters and lecithin, in which a transition from lamellar to cylindrical hexagonal structures was observed. Moreover, monoglycerides and phytosterols have been reported to form complexes due to their ability to interact through hydrogen bonding. However, there was no observable transition in the self-assembly for bi-component of monoglycerides and phytosterols. It has been explained that the self-assembly of a molecule is also determined by its shape, which influences the curvature (bending) angle. In a bi-component (mixed) system, the interaction between host- and guest-molecules influences the curvature angle leading to a change in organization. Thus, the complexation between monoglycerides and phytosterols is speculated incapable to affect the bending angle, which is important to induce a transition in self-assembly structure. Yet, the complexation affects the crystallization of monoglycerides, which is believed to influence the morphology and network of crystals in the bi-component oleogels. Looking into the complex modulus value of the oleogels listed in table 5.2, it shows that the amphiphilic based structurants oleogels had higher complex modulus

than the fully hydrogenated rapeseed oil (TAGs system). This can be explained by the contribution of different non-covalent interactions which is predominantly van der Waals in TAGs crystal, whilst hydrogen bonding and van der Waals forces in amphiphilic based structurants. Thus, it is observed that non-triacylglycerol based structurants found in this study have the potential to provide structuring effect that structure sunflower oil.

The effect of non-covalent interaction on the molecular assembly of combined structurants can be detailed into specific combination. In monoglycerides and phytosterols combination oleogels, it can be postulated that the phytosterols get into the space between the monoglycerides molecules during the formation of inverse lamellar structures. The phytosterols induce chain ordering on the neighboring alkyl tails of monoglycerides. The condensing effect or chain ordering has been widely discussed and proposed in phospholipid membrane containing sterols as the explantion for the improvement in the membrane's physical properties (Cao et al., 2003; de Meyer & Smit, 2009). Specifically, this study postulated the change in the inverse lamellar arrangement, in which shown by the disappearance of peak of 4.11 Å in the 6:4 DM:PSs (Figure 5.8). The effect of phytosterols on the inverse lamellar arrangement of monoglycerides resulted in crystal defect thus change the crystal morphology of the DM:PSs combination oleogels (Figure 3.16 and 3.17). Yet, the actual mechanism of the event led to the crystal defect and the improvement of rheological properties is the subject of future research. As for now, it can be deduced that the change in crystal morphology is responsible for the improvement in the complex modulus. In the sucrose esters-based oleogels, addition of sunflower lecithin induces the change in the self-assembly of the combination oleogels. This was manifested in the transition of the SAXD pattern. Specifically, the 6:4 oleogel exhibited different diffraction pattern than the 10:0 SEs:SFL oleogel. The transition in the molecular organization because both molecules, especially their hydrophilic head, are able to non-covalently bond thus, influencing the curvature angle. The effect on the curvature angle causes transition in the assembly of molecules, organizing into different organization.

Although some potential structurants are insoluble (or partially) in sunflower oil, this problem can be mitigated by using appropriate techniques. In this study, the sucrose esters, methylcellulose, and gelatin were incorporated into sunflower oil through the

indirect approach. Though the HLB of sucrose esters used in this study is 2, direct addition to sunflower oil induced the formation of polydisperse aggregates. These aggregates arise from the monomers with low esterification level such as mono-, di-, and tri-ester that prone to assemble into micelles. Therefore, the incorporation of sucrose esters and its combinations were carefully performed, in which both structurant(s) and sunflower oil should be at 90°C before mixing. This approach manifest that for a potential structurant that prone to aggregate due to entropic hydrophobic effect, addition at high temperature above the melting point helps to mitigate the aggregation. Additionally, it also recommended to add a co-surfactant which helps in dispersing the structurant but also to induce change in the self-assembly. Interestingly, this study used no organic solvent as a bridging agent in the preparation of oleogels. Conventionally, the preparation of stable system in mixed surfactant gel and microemulsion requires the addition of polar solvent to disperse and influencing the bending angle of the molecules. Meanwhile, methylcellulose and gelatin are hydrophilic molecules, in which the formation of network requires certain degree of hydration. Thus, the formation of a network is impossible in sunflower oil. In this study, the incorporation of those hydrophilic polymers was achieved using modified techniques from emulsion-templated approach combined with the approach of producing solid lipid microparticles. The new technique led to production of fat capsules coated with polymer which permits indirect incorporation of methylcellulose and gelatin to sunflower oil by melting the fat capsules. Therefore, it can be said that appropriate technique is required to capitalize the gelling behavior of incompatible structurants in sunflower oil. Interestingly, this study not only introduces new structurants but also introduces oleogelation approach based on the combination of crystalline based and indirect approach categories (crossed category approach) with the fabrication of fat capsules.

The innovative approach of fabrication of polymer coated fat crystals is shown capable to act as a carrier for the hydrophilic polymer to sunflower oil. The approach of fabrication of discrete spherical fat capsules with adsorbed polymer at the surface is a success achievement of this study. Additionally, the spherical morphology and the α-polymorph that analogous to FHRO provide additional advantages to the fabricated fat capsules. The fat capsules exhibit similarity with solid lipid nano/microparticles in term of spherical morphology with internal phase made of fat-based ingredients. However,

most of the fabricated lipid nano/microparticle do not have any stabilizing layer at the surface. The stabilizing layer could act as additional protecting layer to protect the core material for delivery vehicles application of the fat capsules. It is interesting to note that the fabrication process and the polymers did not influence the morphology and the polymorphic type of fat capsules. The fat capsules retained the same polymorphic type as the FHRO, which is in α-polymorph. This is interesting information because the crystallization and polymorphic type of lipid nano/microparticles are important factors to evaluate the efficiency of them as drug carrier (Scalia et al., 2015). In this study, the fat capsules were applied as a structuring agent to gel sunflower oil. During the preparation of oleogels, the fat capsules were heated above their melting temperature to melt the internal fat and hence releasing the hydrophilic polymer to sunflower oil. Upon cooling, the fat, FHRO, recrystallized while the hydrophilic polymer networks randomly entangled in sunflower oil. The presence of polymer networks induced jamming to the fat crystals network thus, improving the rheological properties This approach advances and consolidates the proposed new concept discussed in literature for controlling physical properties of fat crystals network through jamming system (space-filling network or volume effect) (Chauhan et al., 2017a; Patel, 2017; Trappe & Sandkuhler, 2004; Yoshikawa et al., 2015). Ultimately, jamming or volume effect is an interesting physical approach to improve physical properties of fat based systems in addition to interesterification, processing condition, and addition of emulsifiers. The approach to induce jamming can be varied and depending on the introduced molecules or compounds to induce the effect as seen in this study and the studies by Chauhan (2017a and 2017b) and Yoshikawa (2015).

The calorimetry and synchrotron diffraction results presented in this study pertaining to the complexation of monoglycerides and phytosterols provide additional insight on the magnitude of interaction (Akashe & Miller, 2001; Perlman, 2013). In the reported patterns and literature on the monoglycerides and phytosterols complexes, there is no detailed diffraction results provided (Engel & Schubert, 2005; Perlman, 2013). Particularly, this study proved that phytosterols are capable to complex with monoglycerides and hence induce chain ordering. The synchrotron diffraction results, especially in 6:4 (DM:PSs) oleogel during cooling provides new information on the role of phytosterols in affecting the crystallization of monoglycerides (Figure 5.8b). Chen and co-workers (2009) reported the diffraction patterns of monolglycerides and in

agreement with this study. However, the change in the WAXD peak (Figure 5.8b) of monoglycerides in the presence of phytosterols provides additional knowledge. This new knowledge is not only beneficial for food technologist to properly formulate the enrichment of phytosterols in food products, but also for researchers working in biophysical field (lipid membrane). This is particularly important in studying the physical properties of different mesophase structures, for instance liposomes made of monoglycerides in the presence of phytosterols.

Although, a few studies have reported that emulsifiers, for instance monoglyceride, capable to control the crystallization of phytosterols (Engel & Schubert, 2005; von Bonsdorff-Nikander et al., 2003; Zychowski et al., 2016), its function in combination with phytosterols as a structuring unit is a form of innovation. The presence of phytosterols as a co-structuring unit influenced the viscoelastic properties of monoglycerides gel. This study proved that phytosterols is useful ingredient to improve gelation of monoglycerides (distilled monoglycerides) by influencing the crystallization process. Contrary to the approaches reported in the literature in which ethylcellulose (Lopez-Martinez et al., 2015) and shear (Da Pieve et al., 2010) were used to influence the crystallization and physical properties of monoglycerides' oleogels, this study presented an approach which permits simultaneous delivery of a functional ingredient, phytosterols. Currently, phytosterols are added into margarine in their esterified form, as the unesterified form has very high melting point which compromises the quality and functionality of phytosterols (Acevedo & Franchetti, 2016).

The research on microemulsion based on sucrose esters have been widely studied by Garti and co-workers. In most of their study, formation of stable microemulsion requires the addition of co-srufactant such as short chain alcohol (Fanun, Wachtel, et al., 2001; Garti, Aserin, et al., 2000; Garti et al., 1999). This solvent makes the microemulsion has limited application in food product. However, this study investigated the self-assembly of sucrose esters in the presence of lecithin in sunflower oil without added polar solvent. The outcomes of this study clearly manifest the synergistic effect on the self-assembly of sucrose esters by lecithin. Therefore, it is wise to say the finding from this study can be extrapolated to preparation of sucrose esters' microemulsion with lecithin as the substitute for short-chain alcohol. Nonetheless, the preparation of sucrose esters-based oleogels and the combinations were based on mixing at higher temperature, in which the sucrose esters and lecithins readily interact prior to the

addition of sunflower oil. Therefore, it prevented the sucrose esters molecules to aggregate. Interestingly, the formation of sucrose esters and lecithin manifested at concentration lower than other reported oleogels of SAFiNs based.

The approach to characterize and elucidate the synergistic interaction at the nanoscale provides an interesting insight on the interdependency of different length-scales in governing the physical properties of a soft material or gel. In this study, it could be said that the interaction at nanoscale influences the macroscale properties of oleogels. The interdependency of different length-scales has been used to explain the association between crystallization and network of fat to physical properties of fat-based products, as depicted in Figure 1.2. Yet, the properties at the other length-scales, which are not covered in this study, have to be also considered to gain a better picture of the role of synergistic interaction. Nevertheless, the knowledge or information of the nanoscale assembly provides a general picture on the synergistic interaction influencing the assembly or crystallization, particularly for monoglycerides-phytosterols and sucrose esters-lecithins combinations. In the oleogels prepared using fat capsules, no effect on the crystallization of FHRO in the presence of the polymers was detected thus, second the proposed jamming effect in the literature where the space-filling molecules do not affect the crystallization.

The synergistic interactions are the most contributing factor that induce the formation and improvement in the oleogels of oil and structurants systems. The challenge is to retain the structuring mechanism of the respective oleogels in real food systems (application). It has been widely discussed that foods are complex systems containing different phases and ingredients which affect the structuring capability of oleogelators due to matrix effect (Floter, 2012; Mattice & Marangoni, 2017, 2018; McClements et al., 2008). Nevertheless, the outcome of this research especially on the ability of molecules to interact and on the structuring mechanism can be the guidelines for a systematic approach in product application such as a hybrid system. This is to ensure that the structurants are able to attain the same interaction. Failure to attain the interaction causes the combined structurants lose its ability to form an aggregate (structure) and hence structure the oil. Therefore, consideration on the mechanistic formation of gel provided in this study is necessary to successfully use the oleogels in food and other products. Similarly in other applications such as delivery and controlled released (responsive oleogels), the fundamental understanding of the formation and

type of structures (organization) could be used as a basis for a systematic approach in product application. In other words, the studied oleogels represent a simple system and the interaction between the structurants are clear. Therefore, comprehensive understanding of the mechanism of oleogel's formation provides at least systematic guidelines for successful product application. Nonetheless, the next stage of product application also requires extensive and innovative approaches.

Overall, this study focused on the structuring ability of food ingredients (cf: distilled monoglycerides, phytosterols, fully hydrogenated rapeseed oil, sucrose esters, and sunflower lecithin), which contain a mixture of different components. Thus, the outcomes from this study, in general, are beneficial in bridging the understanding between pure components and commercial food ingredients, particularly on the interaction between ingredients that leads to gelation. Currently, there is growing interest among food technologists, especially food physicists, to define the food structuring concept from the soft matter perspective (de Kruif, 2012; Mezzenga et al., 2005; Ubbink, 2012; van der Sman, 2012; Vilgis, 2015). Though the reported results from this study do not involve a complex colloidal calculation and characterization to define the interaction, yet the study provides beneficial results on the structural formation, considering the use of commercial ingredients. Generally, this study helps to bridge the knowledge between pure compounds and commercial ingredients. On one hand, this study outlined the oil structuring properties of combined structurant together with proposed mechanism in sunflower oil. On the other hand, the results of characterization performed on structurants and their combination revealed the interaction. For instance, the effect of phytosterols on the crystallization of monoglycerides (*vice versa*) and the effect of lecithin on the self-assembly behavior of sucrose esters to name a few. These effects merit more fundamental investigation which will be outlined in the following section.

6.2 Future perspectives

6.2.1 Fundamental aspects

The field of edible oleogelation is still in the infancy stage. A considerable number of studies have been conducted in this field particularly on finding edible (GRAS) structurants. The current practice of searching for structurants is based on serendipity and trial and error (Co & Marangoni, 2012). Therefore, it is essential to have a better

approach in selecting potential structurants for edible liquid oil. A possible solution could be integrating the field of oleogelation to the field of supramolecular and colloidal chemistry. Insights from the supramolecular and colloidal chemistry will enable food scientist to design a better performing structurant based on extensive understanding of the structurants' behavior. Consequently, it will provide general guidelines of the characteristic of structurants with high potential to gel edible liquid oil. From this study, specifically the interaction at nanoscale between structurants has an influence to the mesoscale properties of an oleogel. In other words, self-assembly properties of a structurant at nanoscale can be tuned to produce better oleogel. Until now, only the importance of chirality has been acknowledged (Co & Marangoni, 2012; Vintiloiu & Leroux, 2008) which is too general as there is an abundance of molecules with chirality but with weak gelling capacity. In general, it is recommended to have a better integration between the field of chemistry, physics, and food science to gain better understanding of the physical properties of oleogels. Moreover, the integration offers alternative solution to improve the approach of finding edible structurants in the future such as designing functionalized molecules containing side chains that can interact thus, assemble into functionalized architectures.

From the fundamental aspect, it is recommended to investigate the dynamics of self-assembly. This study provides the fundamental understanding of the interfacial properties such as area per molecule and bending/curvature angle. Additionally, it also provides the dimension of self-assembly organization in term of radius, persistence length, and contour length for inverse cylindrical hexagonal structure. Meanwhile, the longitudinal stacking of lamellar, the size of platelets, and the size of crystal flocs for crystalline-based structurants can be refined. These studies can be performed using surface tensiometry, transmission and/or scanning electron microscopy, ultra-small angke X-ray scattering (USAXS), small angle neutron scattering (SANS) and synchrotron x-ray diffraction.

Fabrication of fat capsules, which are identical to solid lipid micro/nanoparticles in pharmaceutical field, requires optimization. The optimization can be performed on the type of machine/equipment that is suitable for the fabrication process, such as microfluidization with temperature control. Additionally, it would be interesting to investigate the absorption capacity of methylcellulose and gelatin at the oil-water interface to obtain the optimum concentration of hydrophilic solution (aqueous phase)

thus, facilitate the fabrication process. The study can be performed by measuring the surface load of corresponding polymers/stabilizer.

In the discussion and conclusion of each chapter in this study, it is hypothesized that non-covalent interactions/bonds have a great influence on the self-assembly and physical properties of network building blocks (Clemente et al., 2012; Fong et al., 2012; Nikiforidis & Scholten, 2014; Wang et al., 2012). Thus, it is of great interest to perform a detailed investigation of the magnitude of non-covalent interactions from a fundamental point of view. This is achievable with advanced instruments such as nuclear magnetic resonance, atomic force microscopy/spectroscopy, Raman spectroscopy, and X-ray diffraction (powder and synchrotron). For instance, Gater and co-workers used nuclear magnetic resonance to quantify the potential of hydrogen bonding between hydroxyl group of sterols to specific locations on the monoglycerides backbone (Gater et al., 2013). The outcome from such study provides a better correlation between the gelling ability of a bi-component system to the contribution from non-covalent interactions.

Additionally, for the bi-component systems of monoglycerides-phytosterols and sucrose esters-lecithin, it is interesting to incorporate water into the systems. It is hypothesized that water will influence the mesophasic behavior (Chen & Terentjev, 2009; Garti, Aserin, et al., 2000; Larsson et al., 1978; Leser et al., 2006; Yaghmur et al., 2006). The most important parameter to consider is the percentage of water that the system can tolerate for a given mesophase structure. The difference in water percentage that the system can tolerate elucidates the role of the guest molecule in controlling the mesophase formation and transition of the host structurant.

Ultimately, it is of great importance to study the effect of preparation steps such as cooling rate, shear, and pressure on the resultant oleogels (Jana & Martini, 2014; Maleky et al., 2012; Moens et al., 2015; Ramel et al., 2016; Sato et al., 2013; Tran & Rousseau, 2016). It has been reported that the processing parameters can help to engineer specific morphologies, thus possibly improving the structuring ability of structurants (Gao et al., 2013; Rogers et al., 2008; Yu et al., 2015). In combination with extensive rheological analysis, the inter-relationship between preparation steps and rheological parameters (*i.e.*, yield stress, viscosity, and flow behavior) can be established.

6.2.2 Application

The proposed suggestions outlined above particularly expand the fundamental understanding of structurants and oleogels. However, the oleogels are also interesting from a practical perspective as they possess solid-like properties and have potential as an alternative to *trans*-fat. Hybrid systems containing both the studied oleogels and existing conventional fats (hardstocks) could be interesting as an approach to manage the level of *trans* and saturated fats in food products. Additionally, it is recommended to mix the oleogels with other gels such as hydrogels to create bigel system that is commonly found in the pharmaceutical industry (Lupi et al., 2015; Lupi, Shakeel, et al., 2016; Patel, Mankoc, et al., 2015). Bigel is a bicontinuous form of emulsion consist of two biphasic systems that the structuring mechanism is based on the independent structuring effect from the respective systems (Patel, Mankoc, et al., 2015). Bigel is proposed due to the lack of information pertaining to behavior of the oleogels in the presence of water, as the preparation of bigel is performed on a solid state of respective gel.

Taking the research's outcomes to a big spectrum and constrating it to the current progress of oleogelation (edible), this research expands the potential of oleogelation field by introducing more edible structurants that are mainly food ingredients. These food ingredients that are introduced as structurants are generally edible. However, the sucrose esters (HLB-2) has limited food application in Europe but allowed in United States of America and Japan. In Europe, only sucrose esters with HLB from 6 and above are allowed to be used in food products. Unfortunately, sucrose esters within the permitted range are not solubilized in sunflower oil and required the assistance of polar solvents (Szuts et al., 2007), wherein this study investigated the properties of water-free oleogels. Interestingly, this study discovered the gelation ability of sucrose esters only in the presence of lecithins thus, limits dependency towards sucrose esters alone for gelation. Therefore, it helps to reduce the concentration of sucrose esters to gel sunflower oil (cf: 10:0 vs 6:4 (SEs:SFL)).

The crystallized oil of fully hydrogenated rapeseed oil as a carrier for hydrophilic polymer provides additional advantages on that ingredient, which is currently used to prevent oiling out (Peyronel et al., 2016). The incorporation of hydrophilic polymers such as methylcellulose or gelatin as in our approach/study helps to influence the

physical properties of end products containing the hydrogenated oil as their ingredient. In order to materialize this application, cooperation with suppliers of fully hydrogenated oil or other crystallized oil is necessary since, preparation of fat capsules has to be done at the first stage of ingredient's preparation. In the field of mechanical and chemical engineering, spherical particles (with average particle size at 1μm) have been used to create capillary suspension (Koos, 2014). This structuring technique is not popular in food science but has bigger potential for exploration using the prepared fat capsules (Chapter 2) for more innovative products, particularly effort to produce colloidal size fat capsules. The large size of fat capsules prepared in this study limits their application in stabilizing colloidal suspension. However, advanced emulsification techniques such as microfluidizer could be employed to fabricate smaller fat capsules. Additionally, delivery of lipophilic functional ingredients is possible using the innovative fabrication approach of this study. Lipophilic ingredients can be incorporated into the fat phase and delivered in the form of fat capsules. This is possible since the fabrication and polymers do not influence the crystallization of internal fat phase. The crystallization and polymorphic form are important parameter to evaluate the efficiency of solid lipid nano/microparticles for drug delivery.

It has been stated previously under the fundamental aspects, addition of water into the oleogels will produce different systems with functionalities applicable for food. For instance, liquid crystal mesophases of monoglycerides (Coasun) has been used as delivery vehicle and as alternative to shortening. Coasun is a zero trans, low-saturated, oil-in-water structured emulsion that functions as a baking margarine prepared from monoglycerides (Wang et al., 2016). Coasun relies on the α-state (gel) of saturated monoglycerides that relied on ionic surfactant to prevent transition to β-state. This study showed the physterols are capable to delay the transition thus, the monoglycerides-phytosterols oleogels pose an advantage to fabricate a product analogous to Coasun. Moreover, similar oleogels based on the combination of monoglycerides and phytosterols have been used for controlled release (responsive oleogel) of volatile compounds (cf: ethyl octanoate) (Yang et al., 2017) and to replace fat in meat products (Kouzounis et al., 2017). Generally, gels from monoglycerides have been widely applied in food, cosmetics and pharmaceutical products as the structuring agent. The advantage of the oleogels developed in this study comes from

the addition of phytosterols as the structuring unit and hence, allowing simultaneous delivery of a phytonutrient in a product.

Sucrose esters-based gels have been used for thermosensitive drug delivery due to their unique liquid mesophase structure, tubular micelles that retain the structure at high temperature (Szuts et al., 2010). Moreover, sucrose esters have been widely used to prepare microemulsion. Currently, the application of sucrose esters in food is only as emulsifier. The formation of gel by sucrose esters in the presence of lecithin introduced in this study could be potentially beneficial to create a responsive oleogel, which responses towards a specific environmental factor (*i.e:* Temperature). Figure 5.14 shows the appearance of diffraction peak in 6:4 (SEs:SFL) oleogel even at temperature above its melting temperature. This result consolidates the proposed application of the oleogels based on sucrose esters and lecithins combination for responsive oleogels. The responsive oleogels can be used to encapsulate ingredients that stabilize even at higher temperature. Similar application has been investigated for pharmaceutical products but with aqueous-based gel of sucrose esters (high HLB) (Chansanroj & Betz, 2010; Szuts et al., 2010). Generally, lipid mesophase structures of surfactants have cutting edge advantages over crystalline-based for the controlled release and drug delivery applications. This is due to the fact that those structures are responsive with better encapsulation properties (Fong et al., 2012; Guo et al., 2010; Kwon & Kim, 2001; O'Sullivan et al., 2016; Sagalowicz, Leser, et al., 2006; Sahoo et al., 2011; Shaikh et al., 2006; Vintiloiu & Leroux, 2008). Additionally, sucrose esters microemulsions have been used as microreactors (Fanun, Leser, Aserin, & Garti, 2001; Garti, Spernath, Aserin, & Lutz, 2005; Lutz, Aserin, & Garti, 2005). Although sucrose esters (low HLB) are not permitted for food application in Europe, there are more potential applications of sucrose esters based oleogels in differents industries.

Additionally, the ability of oleogels' structure (building block) to stabilize air bubbles (oleofoam) has gained considerable attention. This is because for example in the bakery industry, the fat phase is subjected to whipping process to incorporate air into the batter, which is important for the organoleptic properties of resultant cake. Therefore, it is of great interest to whip the oleogels to investigate its capability to stabilize air bubbles. Meanwhile, it is also important to consider that the studied structurants in this study have been used to stabilize air bubbles thus, improving the

chance of creating new product from the oleogels. The whipped oleogels may provide alternative systems to whipped vegetal cream, rich in saturated fat.

As been outlined in the fundamental aspect to study the effect of water on the structures of the oleogels, the proposed applications are restricted to water-free system (*i.e*: shortening). Majority of the structurants found in this study are emulsifiers, which are surface active (reactive). This property makes the process to upscale the oleogels to real system challenging. The challenge is to keep the structurants to retain their functionality to provide structural building blocks with less interference from surrounding environments, especially water. Although in water-free system such as shortening, majority of the structurants have an effect on crystallization of hard-stock. Thus, formation of hybrid system or bigel requires extensive optimization in the processing parameters such as mixing temperature (thermal behavior), ratio of oleogel to hardstock, and mixing speed.

7 Appendix

Table A1 Composition of fully hydrogenated rapeseed oil (Sources: Supplier and (G. Y. Kim & Marangoni, 2017)

Content	Percentage (%)
Fatty acid composition	
Palmitic acid	4.5
Stearic acid	40.8
Arachidic acid	10.2
Behenic acid	42.8
TAG composition	
PPS	2.5
PSS	7.7
SSS	16.7
BSP	21.2
SSB	19.9
BSA	17.4
BBS	14.6
Carbon number (TAG)	
48	0.3
50	1.4
52	3.3
54	9.1
56	9.5
58	13.1
60	19.0
62	40
64	1.8

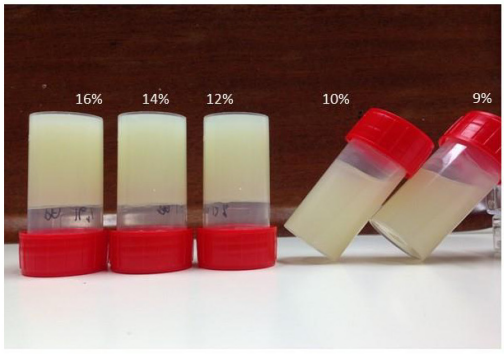

Figure A1 Determination of minimum gelling concentration of Fully hydrogenated rapeseed oil (FHRO) in sunflower oil.

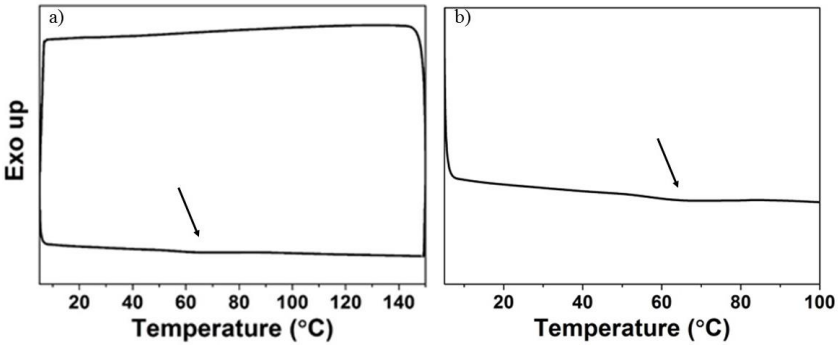

Figure A2 The crystallization and melting profiles of 10% wt PSs in sunflower oil. The melting profile was obtained after one-hour crystallization at 5°C.

Table A2 The peak melting temperatures (T_{mp}) and melting enthalpy (H_{mp}) of the distilled monoglycerides (DM):Phytosterols (PSs) oleogels at different crystallization periods (N=3 of independent sample).

Oleogels (DM:PSs)	One-hour		Three-hour		One-week	
	T_{mp} (°C)	H_{mp} (J/g)	T_{mp} (°C)	H_{mp} (J/g)	T_{mp} (°C)	H_{mp} (J/g)
10:0	54.23 ±0.33^{bA}	7.84 ±0.41^{eA}	54.23 ±0.39^{bA}	10.06 ±0.57^{eAB}	55.67 ±0.37^{aB}	11.53 ±1.72^{dB}
9:1	54.76 ±0.26^{bA}	6.41 ±0.43^{dA}	54.69 ±0.10^{bA}	7.06 ±0.41^{dA}	55.92 ±0.10^{aB}	9.27 ±0.40^{cB}
8:2	54.48 ±0.67^{bA}	3.31 ±0.09^{cA}	53.26 ±0.82^{abA}	4.66 ±0.13^{cA}	53.63 ±1.82^{aA}	7.97 ±0.76^{cB}
6:4	36.68 ±0.62^{aA}	2.74 ±0.08^{bA}	51.25 ±0.05^{aB}	3.54 ±0.22^{bA}	51.04 ±2.52^{aB}	7.47 ±0.51^{cB}
6:0	54.30 ±0.33^{bA}	2.79 ±0.15^{cbA}	54.16 ±0.53^{bA}	4.47 ±0.36^{cB}	54.15 ±0.20^{aA}	5.32 ±0.10^{bC}
0:10	69.59 ±2.33^{cA}	1.03 ±0.06^{aA}	71.06 ±2.23^{cA}	1.08 ±0.13^{aA}	73.46 ±4.77^{bA}	1.98 ±0.03^{aB}

Small letter = indicative the significant difference (P<0.01) between the oleogels in the same colum
Capital letter = Indicative the significant difference (P<0.01) of the same oleogel on the same parameter at different crystallization period (Row)

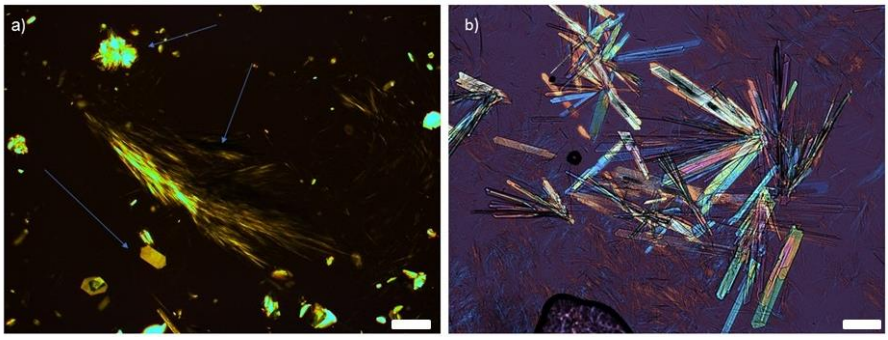

Figure A3 The crystal morphologies of phytosterols (PS) in sunflower oil at 5wt% (a) and 1 wt% (b) of total concentration. The scale bar is 100μm.

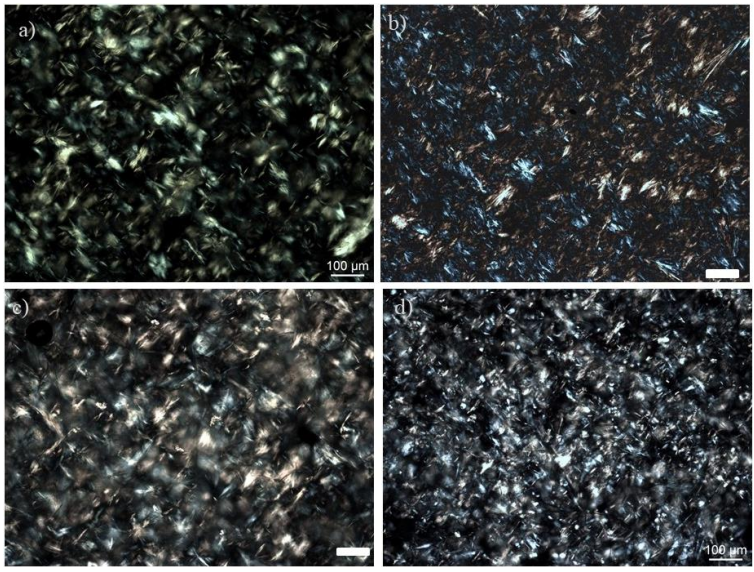

Figure A4 The morphology of crystals of 10:0 (a), 9:1 (b), 8:2 (c), and 6:4 (d) DM:PSs oleogels. The samples were taken directly from the oleogels. The scale bar is 100μm.

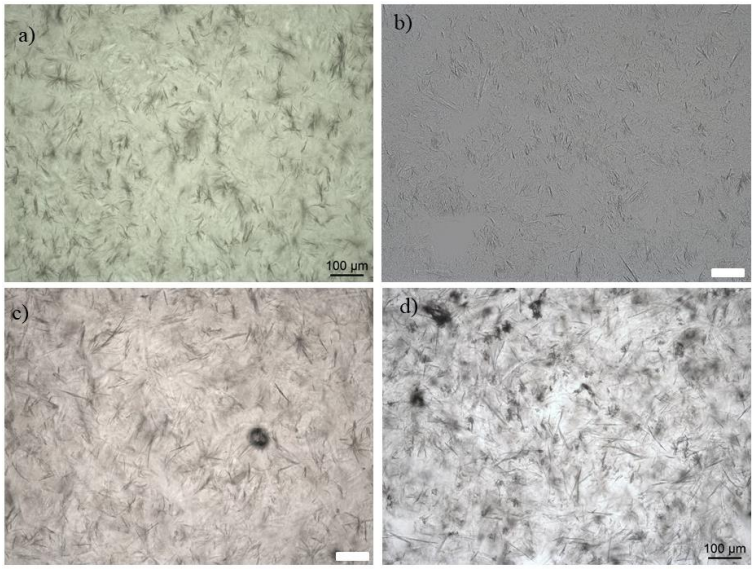

Figure A5 The optical light microscopy images of the 10:0 (a), 9:1 (b), 8:2 (c), and 6:4 (d) DM:PSs oleogels. The samples were taken directly from the oleogels. The scale bar is 100μm.

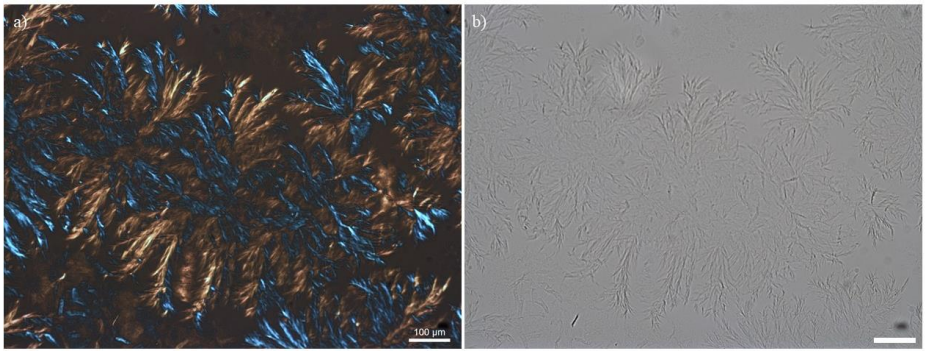

Figure A6 The morphology of crystals of 6:0 DM:PSs visualized using polarized (a) and optical light microscopy (b). The scale bar is 100µm.

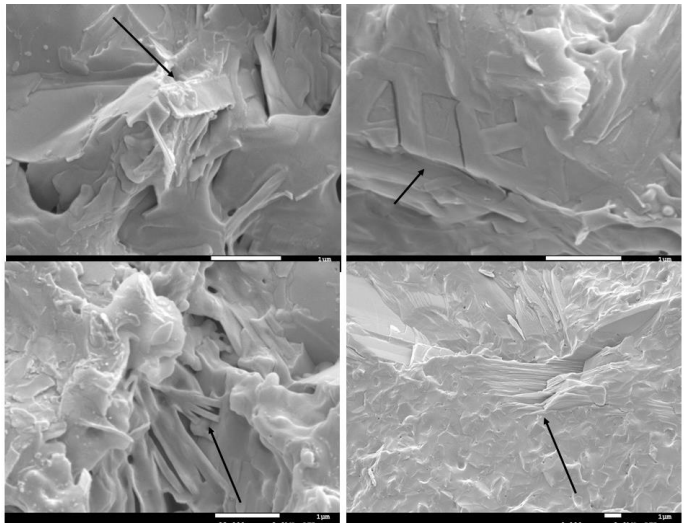

Figure A7 The internal structure of 6:4 DM:PSs oleogel. The oleogel was freeze-fractured to visualize the network structure.

Table A3 The peak melting temperatures (T_{mp}), melting enthalpy (H_{mp}), and offset melting temperature (T_{off}) of the oleogels at different crystallization periods (t-test) (N=3 of independent samples).

Oleogels (SEs:SFL)	Three-hour			One-week		
	T_{mp} (°C)	H_{mp} (J/g)	T_{off} (°C)	T_{mp} (°C)	H_{mp} (J/g)	T_{off} (°C)
10:0	65.82±0.06^{dA}	7.28 ±0.15^{cA}	73.16 ±2.15^{bA}	65.01 ±0.56^{cA}	8.38 ±0.49^{dB}	74.29 ±0.52^{cA}
8:2	61.89 ±0.23^{cA}	6.87 ±0.25^{cA}	73.70 ±0.71^{bA}	53.45 ±0.34^{bB}	7.39 ±0.13^{cB}	72.68 ±1.06^{cA}
7:3	60.17 ±0.23^{bA}	6.18 ±1.06^{bA}	70.31 ±0.40^{bA}	53.43 ±0.07^{bB}	6.09 ±0.04^{bA}	68.40 ±0.49^{bB}
6:4	53.56 ±0.96^{aA}	4.54 ±0.37^{aA}	65.64 ±0.70^{aA}	52.12 ±0.16^{aA}	5.12 ±0.37^{aA}	65.20 ±0.81^{aA}

Small letter = indicative the significant difference (P<0.01) between the oleogels in the same colum
Capital letter= Indicative the significant difference (P<0.01) of the same oleogel on the same parameter at different crystallization period (Row)

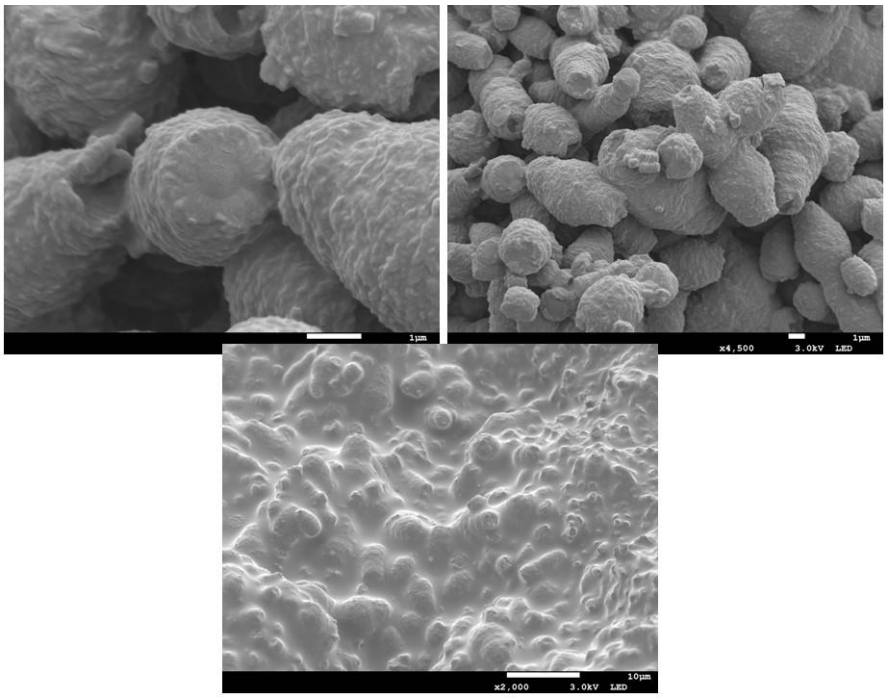

Figure A8 The morphology of sucrose esters (SEs) mono-component at 10 wt% concentration in sunflower oil. The liquid oil was extracted out using hexane solvent.

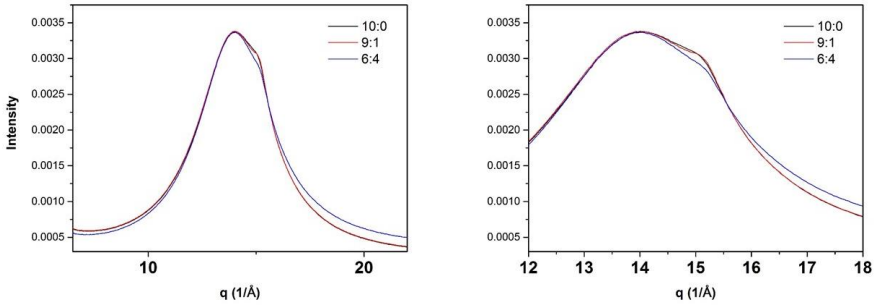

Figure A9 The WAXD pattern of oleogels prepared from the combination of sucrose esters and lecithin (SEs:SFL) in sunflower oil obtained using synchrotron radiation.

8 Bibliography

Abdallah, D. J., Sirchio, S. A., & Weiss, R. G. (2000). Hexatriacontane organogels. The first determination of the conformation and molecular packing of a low-molecular-mass organogelator in its gelled state. *Langmuir, 16* (20), 7558-7561.

Abdallah, D. J., & Weiss, R. G. (2000). Organogels and low molecular mass organic gelators. *Advanced Materials, 12* (17), 1237-+.

Acevedo, N. C., Block, J. M., & Marangoni, A. G. (2012a). Critical laminar shear-temperature effects on the nano- and mesoscale structure of a model fat and its relationship to oil binding and rheological properties. *Faraday Discussions, 158*, 171-194.

Acevedo, N. C., Block, J. M., & Marangoni, A. G. (2012b). Unsaturated Emulsifier-Mediated Modification of the Mechanical Strength and Oil Binding Capacity of a Model Edible Fat Crystallized under Shear. *Langmuir, 28* (46), 16207-16217.

Acevedo, N. C., & Franchetti, D. (2016). Analysis of co-crystallized free phytosterols with triacylglycerols as a functional food ingredient. *Food Research International, 85*, 104-112.

Acevedo, N. C., & Marangoni, A. G. (2010). Characterization of the Nanoscale in Triacylglycerol Crystal Networks. *Crystal Growth & Design, 10* (8), 3327-3333.

Acevedo, N. C., & Marangoni, A. G. (2015). Nanostructured Fat Crystal Systems. *Annual Review of Food Science and Technology, Vol 6, 6*, 71-96.

Acevedo, N. C., Peyronel, F., & Marangoni, A. G. (2011). Nanoscale structure intercrystalline interactions in fat crystal networks. *Current Opinion in Colloid & Interface Science, 16* (5), 374-383.

Acharya, D. P., Varade, D., & Aramaki, K. (2007). Effect of temperature on the rheology of wormlike micelles in a mixed surfactant system. *Journal of Colloid and Interface Science, 315* (1), 330-336.

Akashe, A., & Miller, M. (2001). Plant sterol-emulsifier complexes. In. United States: Kraft Foods, Inc.

Arboleya, J. C., & Wilde, P. J. (2005). Competitive adsorption of proteins with methylcellulose and hydroxypropyl methylcellulose. *Food Hydrocolloids, 19* (3), 485-491.

Arleth, L., Bauer, R., Ogendal, L. H., Egelhaaf, S. U., Schurtenberger, P., & Pedersen, J. S. (2003). Growth behavior of mixed wormlike micelles: a small-angle scattering study of the lecithin-bile salt system. *Langmuir, 19* (10), 4096-4104.

Aro, A., Jauhiainen, M., Partanen, R., Salminen, I., & Mutanen, M. (1997). Stearic acid, trans fatty acids, and dairy fat: Effects on serum and lipoprotein lipids, apolipoproteins, lipoprotein(a), and lipid transfer proteins in healthy subjects. *American Journal of Clinical Nutrition, 65* (5), 1419-1426.

Asperger, A., Engewald, W., & Fabian, G. (1999). Analytical characterization of natural waxes employing pyrolysis-gas chromatography-mass spectrometry. *Journal of Analytical and Applied Pyrolysis, 50* (2), 103-115.

Bach, D., & Wachtel, E. (2003). Phospholipid/cholesterol model membranes: formation of cholesterol crystallites. *Biochimica Et Biophysica Acta-Biomembranes, 1610* (2), 187-197.

Barbut, S., Wood, J., & Marangoni, A. (2016a). Potential use of organogels to replace animal fat in comminuted meat products. *Meat Science, 122*, 155-162.

Barbut, S., Wood, J., & Marangoni, A. (2016b). Quality effects of using organogels in breakfast sausage. *Meat Science, 122*, 84-89.

Barbut, S., Wood, J., & Marangoni, A. G. (2016c). Effects of Organogel Hardness and Formulation on Acceptance of Frankfurters. *Journal of Food Science, 81* (9), C2183-C2188.

Bayes-Garcia, L., Patel, A. R., Dewettinck, K., Rousseau, D., Sato, K., & Ueno, S. (2015). Lipid crystallization kinetics - roles of external factors influencing functionality of end products. *Current Opinion in Food Science, 4*, 32-38.

Becerra, N., Toro, C., Zanocco, A. L., Lemp, E., & Gunther, G. (2008). Characterization of micelles formed by sucrose 6-O-monoesters. *Colloids and Surfaces a-Physicochemical and Engineering Aspects, 327* (1-3), 134-139.

Bin Sintang, M. D., Rimaux, T., Van de Walle, D., Dewettinck, K., & Patel, A. R. (2017). Oil structuring properties of monoglycerides and phytosterols mixtures. *European Journal of Lipid Science and Technology, 119* (3), 1500517.

Binks, B. P., & Marinopoulos, I. (2017). Ultra-stable self-foaming oils. *Food Research International, 95*, 28-37.

Blach, C., Gravelle, A. J., Peyronel, F., Weiss, J., Barbut, S., & Marangoni, A. G. (2016). Revisiting the crystallization behavior of stearyl alcohol : stearic acid (SO : SA) mixtures in edible oil. *Rsc Advances, 6* (84), 81151-81163.

Blake, A. I., & Marangoni, A. G. (2015). The Use of Cooling Rate to Engineer the Microstructure and Oil Binding Capacity of Wax Crystal Networks. *Food Biophysics, 10* (4), 456-465.

Bodennec, M., Guo, Q., & Rousseau, D. (2016). Molecular and microstructural characterization of lecithin-based oleogels made with vegetable oil. *RSC Adv., 6* (53), 47373-47381.

Bolzinger-Thevenin, M. A., Grossiord, J. L., & Poelman, M. C. (1999). Characterization of a sucrose ester microemulsion by freeze fracture electron micrograph and small angle neutron scattering experiments. *Langmuir, 15* (7), 2307-2315.

Bossler, F., & Koos, E. (2016). Structure of Particle Networks in Capillary Suspensions with Wetting and Nonwetting Fluids. *Langmuir, 32* (6), 1489-1501.

Bot, A., & Agterof, W. G. M. (2006). Structuring of edible oils by mixtures of gamma-oryzanol with beta-sitosterol or related phytosterols. *Journal of the American Oil Chemists Society, 83* (6), 513-521.

Bot, A., den Adel, R., & Roijers, E. C. (2008). Fibrils of gamma-Oryzanol plus beta-Sitosterol in Edible Oil Organogels. *Journal of the American Oil Chemists Society, 85* (12), 1127-1134.

Bot, A., den Adel, R., Roijers, E. C., & Regkos, C. (2009). Effect of Sterol Type on Structure of Tubules in Sterol plus gamma-Oryzanol-Based Organogels. *Food Biophysics, 4* (4), 266-272.

Bot, A., & Flöter, E. (2011). Edible Oil Organogels Based on Self-assembled β-sitosterol + γ-oryzanol Tubules. 49-79.

Bot, A., Gilbert, E. P., Bouwman, W. G., Sawalha, H., den Adel, R., Garamus, V. M., Venema, P., van der Linden, E., & Floter, E. (2012). Elucidation of density profile of self-assembled sitosterol plus oryzanol tubules with small-angle neutron scattering. *Faraday Discussions, 158*, 223-238.

Bot, A., Veldhuizen, Y. S. J., den Adel, R., & Roijers, E. C. (2009). Non-TAG structuring of edible oils and emulsions. *Food Hydrocolloids, 23* (4), 1184-1189.

Botega, D. C. Z., Marangoni, A. G., Smith, A. K., & Goff, H. D. (2013). Development of Formulations and Processes to Incorporate Wax Oleogels in Ice Cream. *Journal of Food Science, 78* (12), C1845-C1851.

Boyd, B. J., Khoo, S. M., Whittaker, D. V., Davey, G., & Porter, C. J. H. (2007). A lipid-based liquid crystalline matrix that provides sustained release and enhanced oral bioavailability for a model poorly water soluble drug in rats. *International Journal of Pharmaceutics, 340* (1-2), 52-60.

Buerkle, L. E., & Rowan, S. J. (2012). Supramolecular gels formed from multi-component low molecular weight species. *Chemical Society Reviews, 41* (18), 6089-6102.

Calligaris, S., Valoppi, F., Barba, L., Anese, M., & Nicoli, M. C. (2014). Mutual effect of fat and beta-carotene on fat crystal network structure and carotenoid bleaching. *Food Research International, 66*, 257-263.

Cao, H. H., Tokutake, N., & Regen, S. L. (2003). Unraveling the mystery surrounding cholesterol's condensing effect. *Journal of the American Chemical Society, 125* (52), 16182-16183.

Cardiel, J. J., Furusho, H., Skoglund, U., & Shen, A. Q. (2015). Formation of crystal-like structures and branched networks from nonionic spherical micelles. *Scientific Reports, 5*.

Cerqueira, M. A., Fasolin, L. H., Picone, C. S. F., Pastrana, L. M., Cunha, R. L., & Vicente, A. A. (2017). Structural and mechanical properties of organogels: Role of oil and gelator molecular structure. *Food Research International, 96* (96), 161-170.

Chansanroj, K., & Betz, G. (2010). Sucrose esters with various hydrophilic-lipophilic properties: Novel controlled release agents for oral drug delivery matrix tablets prepared by direct compaction. *Acta Biomaterialia, 6* (8), 3101-3109.

Chauhan, R. R., Dullens, R. P., Velikov, K. P., & Aarts, D. G. (2017a). The effect of colloidal aggregates on fat crystal networks. *Food & Function, 8* (1), 352-359.

Chauhan, R. R., Dullens, R. P. A., Velikov, K. P., & Aarts, D. G. A. L. (2017b). Exploring concentration, surface area and surface chemistry effects of colloidal aggregates on fat crystal networks. *Rsc Advances, 7* (46), 28780-28787.

Chen, C. H., & Terentjev, E. M. (2009). Aging and metastability of monoglycerides in hydrophobic solutions. *Langmuir, 25* (12), 6717-6724.

Chen, C. H., Zhang, H., Bi, Y. L., & Cheong, L. Z. (2015). Effects of Sucrose Esters on Isothermal Crystallization of Palm Oil-based Blend. *Journal of the American Oil Chemists Society, 92* (2), 277-286.

Chen, D. T. N., Wen, Q., Janmey, P. A., Crocker, J. C., & Yodh, A. G. (2010). Rheology of Soft Materials. *Annual Review of Condensed Matter Physics, Vol 1, 1*, 301-322.

Cheng, H. H., Lu, C., Liu, J. J., Yan, Y. K., Han, X. B., Jin, H. M., Wang, Y., Liu, Y., & Wu, C. L. (2017). Synchrotron radiation X-ray powder diffraction techniques applied in hydrogen storage materials - A review. *Progress in Natural Science-Materials International, 27* (1), 72-79.

Christiansen, L. I., Rantanen, J. T., von Bonsdorff, A. K., Karjalainen, M. A., & Yliruusi, J. K. (2002). A novel method of producing a microcrystalline beta-sitosterol suspension in oil. *European Journal of Pharmaceutical Sciences, 15* (3), 261-269.

Clemente, M. J., Romero, P., Serrano, J. L., Fitremann, J., & Oriol, L. (2012). Supramolecular Hydrogels Based on Glycoamphiphiles: Effect of the Disaccharide Polar Head. *Chemistry of Materials, 24* (20), 3847-3858.

Co, E. D., & Marangoni, A. G. (2012). Organogels: An Alternative Edible Oil-Structuring Method. *Journal of the American Oil Chemists Society, 89* (5), 749-780.

Coupland, J. N. (2002). Crystallization in emulsions. *Current Opinion in Colloid & Interface Science, 7* (5-6), 445-450.

Craven, B. M. (1976). Crystal structure of cholesterol monohydrate. *Nature, 260* (5553), 727-729.

Crilly, J. F., & Earnshaw, J. C. (1983). Cholesterol-Induced Effects on the Viscoelasticity of Monoglyceride Bilayers. *Biophysical Journal, 41* (2), 211-216.

Da Pieve, S., Calligaris, S., Co, E., Nicoli, M. C., & Marangoni, A. G. (2010). Shear Nanostructuring of Monoglyceride Organogels. *Food Biophysics, 5* (3), 211-217.

Da Pieve, S., Calligaris, S., Panozzo, A., Arrighetti, G., & Nicoli, M. C. (2011). Effect of monoglyceride organogel structure on cod liver oil stability. *Food Research International, 44* (9), 2978-2983.

Daniel, J., & Rajasekharan, R. (2003). Organogelation of plant oils and hydrocarbons by long-chain saturated FA, fatty alcohols, wax esters, and dicarboxylic acids. *Journal of the American Oil Chemists Society, 80* (5), 417-421.

Danthine, S., & Deroanne, C. (2003). Blending of hydrogenated low-erucic acid rapeseed oil, low-erucic acid rapeseed oil, and hydrogenated palm oil or palm oil in the preparation of shortenings. *Journal of the American Oil Chemists Society, 80* (11), 1069-1075.

Dassanayake, L. S. K., Kodali, D. R., & Ueno, S. (2011). Formation of oleogels based on edible lipid materials. *Current Opinion in Colloid & Interface Science, 16* (5), 432-439.

Dastidar, P. (2008). Supramolecular gelling agents: can they be designed? *Chemical Society Reviews, 37* (12), 2699-2715.

Davidovich-Pinhas, M., Barbut, S., & Marangoni, A. G. (2015). The gelation of oil using ethyl cellulose. *Carbohydrate Polymers, 117*, 869-878.

Davidovich-Pinhas, M., Barbut, S., & Marangoni, A. G. (2016). Development, Characterization, and Utilization of Food-Grade Polymer Oleogels. *Annual Review of Food Science and Technology, Vol 7, 7*, 65-91.

Davidovich-Pinhas, M., Gravelle, A. J., Barbut, S., & Marangoni, A. G. (2015). Temperature effects on the gelation of ethylcellulose oleogels. *Food Hydrocolloids, 46*, 76-83.

De Colli, M., Massimi, M., Barbetta, A., Di Rosario, B. L., Nardecchia, S., Devirgiliis, L. C., & Dentini, M. (2012). A biomimetic porous hydrogel of gelatin and glycosaminoglycans cross-linked with transglutaminase and its application in the culture of hepatocytes. *Biomedical Materials, 7* (5).

De Graef, V., Goderis, B., Van Puyvelde, P., Foubert, I., & Dewettinck, K. (2008). Development of a rheological method to characterize palm oil crystallizing under shear. *European Journal of Lipid Science and Technology, 110* (6), 521-529.

de Kruif, C. G. (2012). Concluding remarks: the future of soft matter and food structure. *Faraday Discussions, 158*, 523-527.

de Meyer, F., & Smit, B. (2009). Effect of cholesterol on the structure of a phospholipid bilayer. *Proceedings of the National Academy of Sciences of the United States of America, 106* (10), 3654-3658.

de Vries, A., Hendriks, J., van der Linden, E., & Scholten, E. (2015). Protein Oleogels from Protein Hydrogels via a Stepwise Solvent Exchange Route. *Langmuir, 31* (51), 13850-13859.

de Vries, A., Lopez Gomez, Y., Jansen, B., van der Linden, E., & Scholten, E. (2017). Controlling Agglomeration of Protein Aggregates for Structure Formation in Liquid Oil: A Sticky Business. *ACS Appl Mater Interfaces, 9* (11), 10136-10147.

de Vries, A., Wesseling, A., van der Linden, E., & Scholten, E. (2017). Protein oleogels from heat-set whey protein aggregates. *Journal of Colloid and Interface Science, 486*, 75-83.

Delacharlerie, S., Petrut, R., Deckers, S., Floter, E., Blecker, C., & Danthine, S. (2016). Structuring effects of lecithins on model fat systems: A comparison between native and hydrolyzed forms. *Lwt-Food Science and Technology, 72*, 552-558.

Deman, J. M. (1992). X-Ray-Diffraction Spectroscopy in the Study of Fat Polymorphism. *Food Research International, 25* (6), 471-476.

Deman, L., Deman, J. M., & Blackman, B. (1989). Polymorphic Behavior of Some Fully Hydrogenated Oils and Their Mixtures with Liquid Oil. *Journal of the American Oil Chemists Society, 66* (12), 1777-1780.

Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids, 17* (1), 25-39.

Dickinson, E. (2009). Hydrocolloids as emulsifiers and emulsion stabilizers. *Food Hydrocolloids, 23* (6), 1473-1482.

Dickinson, E., Stainsby, G., & Wilson, L. (1985). An Adsorption Effect on the Gel Strength of Dilute Gelatin-Stabilized Oil-in-Water Emulsions. *Colloid and Polymer Science, 263* (11), 933-934.

Doan, C. D., Patel, A. R., Tavernier, I., De Clercq, N., Van Raemdonck, K., Van de Walle, D., Delbaere, C., & Dewettinck, K. (2016). The feasibility of wax-based oleogel as a potential co-structurant with palm oil in low-saturated fat confectionery fillings. *European Journal of Lipid Science and Technology, 118* (12), 1903-1914.

Doan, C. D., Tavernier, I., Bin Sintang, M. D., Danthine, S., Van de Walle, D., Rimaux, T., & Dewettinck, K. (2017). Crystallization and Gelation Behavior of Low- and High Melting Waxes in Rice Bran Oil: a Case-Study on Berry Wax and Sunflower Wax. *Food Biophysics, 12* (1), 97-108.

Doan, C. D., Van de Walle, D., Dewettinck, K., & Patel, A. R. (2015). Evaluating the Oil-Gelling Properties of Natural Waxes in Rice Bran Oil: Rheological, Thermal, and Microstructural Study. *Journal of the American Oil Chemists Society, 92* (6), 801-811.

Douaire, M., di Bari, V., Norton, J. E., Sullo, A., Lillford, P., & Norton, I. T. (2014). Fat crystallisation at oil-water interfaces. *Advances in Colloid and Interface Science, 203*, 1-10.

Draper, E. R., & Adams, D. J. (2017). Low-Molecular-Weight Gels: The State of the Art. *Chem, 3* (3), 390-410.

Duffy, N., Blonk, H. C. G., Beindorff, C. M., Cazade, M., Bot, A., & Duchateau, G. S. M. J. E. (2009). Organogel-Based Emulsion Systems, Micro-Structural Features and Impact on In Vitro Digestion. *Journal of the American Oil Chemists Society, 86* (8), 733-741.

Engel, R., & Schubert, H. (2005). Formulation of phytosterols in emulsions for increased dose response in functional foods. *Innovative Food Science & Emerging Technologies, 6* (2), 233-237.

Engelskirchen, S., Acharya, D. P., Garcia-Roman, M., & Kunieda, H. (2006). Effect of C12EOn mixed surfactant systems on the formation of viscoelastic worm-like micellar solutions in sucrose alkanoate- and CTAB-water systems. *Colloids and Surfaces a-Physicochemical and Engineering Aspects, 279* (1-3), 113-120.

Fameau, A. L., Lam, S., Arnould, A., Gaillard, C., Velev, O. D., & Saint-Jalmes, A. (2015). Smart Nonaqueous Foams from Lipid-Based Oleogel. *Langmuir, 31* (50), 13501-13510.

Fameau, A. L., & Saint-Jalmes, A. (2017). Non-aqueous foams: Current understanding on the formation and stability mechanisms. *Adv Colloid Interface Sci*.

Fanun, M., Leser, M., Aserin, A., & Garti, N. (2001). Sucrose ester microemulsions as microreactors for model Maillard reaction. *Colloids and Surfaces a-Physicochemical and Engineering Aspects, 194* (1-3), 175-187.

Fanun, M., Wachtel, E., Antalek, B., Aserin, A., & Garti, N. (2001). A study of the microstructure of four-component sucrose ester microemulsions by SAXS and NMR. *Colloids and Surfaces a-Physicochemical and Engineering Aspects, 180* (1-2), 173-186.

Fischer, P., Pollard, M., Erni, P., Marti, I., & Padar, S. (2009). Rheological approaches to food systems. *Comptes Rendus Physique, 10* (8), 740-750.

Fischer, P., & Windhab, E. J. (2011). Rheology of food materials. *Current Opinion in Colloid & Interface Science, 16* (1), 36-40.

Floter, E. (2012). Structuring oils without highly saturated fats - how far are we? *European Journal of Lipid Science and Technology, 114* (9), 983-984.

Floury, J., Desrumaux, A., Axelos, M. A. V., & Legrand, J. (2003). Effect of high pressure homogenisation on methylcellulose as food emulsifier. *Journal of Food Engineering, 58* (3), 227-238.

Fong, C., Le, T., & Drummond, C. J. (2012). Lyotropic liquid crystal engineering-ordered nanostructured small molecule amphiphile self-assembly materials by design. *Chemical Society Reviews, 41* (3), 1297-1322.

Foubert, I., Dewettinck, K., Janssen, G., & Vanrolleghem, P. A. (2006). Modelling two-step isothermal fat crystallization. *Journal of Food Engineering, 75* (4), 551-559.

Foubert, I., Fredrick, E., Vereecken, J., Sichien, M., & Dewettinck, K. (2008). Stop-and-return DSC method to study fat crystallization. *Thermochimica Acta, 471* (1-2), 7-13.

Gandolfo, F. G., Bot, A., & Floter, E. (2004). Structuring of edible oils by long-chain FA, fatty alcohols, and their mixtures. *Journal of the American Oil Chemists Society, 81* (1), 1-6.

Gao, J., Wu, S., Emge, T. J., & Rogers, M. A. (2013). Nanoscale and microscale structural changes alter the critical gelator concentration of self-assembled fibrillar networks. *Crystengcomm, 15* (22), 4507-4515.

Garbolino, C., Bartoccini, M., & Floter, E. (2005). The influence of emulsifiers on the crystallisation behaviour of a palm oil-based blend. *European Journal of Lipid Science and Technology, 107* (9), 616-626.

Garti, N., Aserin, A., & Fanun, M. (2000). Non-ionic sucrose esters microemulsions for food applications. Part 1. Water solubilization. *Colloids and Surfaces a-Physicochemical and Engineering Aspects, 164* (1), 27-38.

Garti, N., Clement, V., Fanun, M., & Leser, M. E. (2000). Some characteristics of sugar ester nonionic microemulsions in view of possible food applications. *Journal of Agricultural and Food Chemistry, 48* (9), 3945-3956.

Garti, N., Clement, V., Leser, M., Aserin, A., & Fanun, M. (1999). Sucrose ester microemulsions. *Journal of molecular liquids, 80* (2-3), 253-296.

Garti, N., Spernath, A., Aserin, A., & Lutz, R. (2005). Nano-sized self-assemblies of nonionic surfactants as solubilization reservoirs and microreactors for food systems. *Soft Matter, 1* (3), 206-218.

Gater, D. L., Reat, V., Czaplicki, G., Saurel, O., Milon, A., Jolibois, F., & Cherezov, V. (2013). Hydrogen Bonding of Cholesterol in the Lipidic Cubic Phase. *Langmuir, 29* (25), 8031-8038.

Gennes, P.-G. d. (2005). Soft matter: more than words. *Soft Matter, 1* (1), 16.

German, J. B., & Dillard, C. J. (2004). Saturated fats: what dietary intake? *American Journal of Clinical Nutrition, 80* (3), 550-559.

Gilbert, E. P., Lopez-Rubio, A., & Gidley, M. J. (2015). Characterisation techniques in food materials science. In B. Bhandari & Y. H. Roos (Eds.), *Food Materials Science and Engineering* (pp. 52-93). United Kingdom: Wiley-Blackwell.

Gilsenan, P. M., & Ross-Murphy, S. B. (2000). Rheological characterisation of gelatins from mammalian and marine sources. *Food Hydrocolloids, 14* (3), 191-195.

Glampedaki, P., & Dutschk, V. (2014). Stability studies of cosmetic emulsions prepared from natural products such as wine, grape seed oil and mastic resin. *Colloids and Surfaces a-Physicochemical and Engineering Aspects, 460*, 306-311.

Gravelle, A. J., Davidovich-Pinhas, M., Barbut, S., & Marangoni, A. G. (2017). Influencing the crystallization behavior of binary mixtures of stearyl alcohol and stearic acid (SOSA) using ethylcellulose. *Food Research International, 91*, 1-10.

Gravelle, A. J., Davidovich-Pinhas, M., Zetzl, A. K., Barbut, S., & Marangoni, A. G. (2016). Influence of solvent quality on the mechanical strength of ethylcellulose oleogels. *Carbohydrate Polymers, 135*, 169-179.

Gunes, D. Z., Murith, M., Godefroid, J., Pelloux, C., Deyber, H., Schafer, O., & Breton, O. (2017). Oleofoams: Properties of Crystal-Coated Bubbles from Whipped Oleogels-Evidence for Pickering Stabilization. *Langmuir, 33* (6), 1563-1575.

Guo, C. Y., Wang, J., Cao, F. L., Lee, R. J., & Zhai, G. X. (2010). Lyotropic liquid crystal systems in drug delivery. *Drug Discovery Today, 15* (23-24), 1032-1040.

Guo, Q., Ye, A., Bellissimo, N., Singh, H., & Rousseau, D. (2017). Modulating fat digestion through food structure design. *Progress in Lipid Research, 68*, 109-118.

Hagemann, J. W., & Rothfus, J. A. (1983). Polymorphism and Transformation Energetics of Saturated Monoacid Triglycerides from Differential Scanning Calorimetry and Theoretical Modeling. *Journal of the American Oil Chemists Society, 60* (6), 1123-1131.

Halling, K. K., & Slotte, J. P. (2004). Membrane properties of plant sterols in phospholipid bilayers as determined by differential scanning calorimetry, resonance energy transfer and detergent-induced solubilization. *Biochimica Et Biophysica Acta-Biomembranes, 1664* (2), 161-171.

Hammond, M. R., & Mezzenga, R. (2008). Supramolecular routes towards liquid crystalline side-chain polymers. *Soft Matter, 4* (5), 952-961.

Han, L. J., Li, L., Li, B., Zhao, L., Liu, G. Q., Liu, X. Q., & Wang, X. D. (2014). Structure and Physical Properties of Organogels Developed by Sitosterol and Lecithin with Sunflower Oil. *Journal of the American Oil Chemists Society, 91* (10), 1783-1792.

Hanani, Z. A. N., Roos, H., & Kerry, J. P. (2012). Use of beef, pork and fish gelatin sources in the manufacture of films and assessment of their composition and mechanical properties. *Food Hydrocolloids, 29* (1), 144-151.

Haque, A., & Morris, E. R. (1993). Thermogelation of Methylcellulose .1. Molecular-Structures and Processes. *Carbohydrate Polymers, 22* (3), 161-173.

Hashizaki, K., Taguchi, H., & Saito, Y. (2009). A novel reverse worm-like micelle from a lecithin/sucrose fatty acid ester/oil system. *Colloid and Polymer Science, 287* (9), 1099-1105.

Haug, I. J., Draget, K. I., & Smidsrod, A. (2004). Physical and rheological properties of fish gelatin compared to mammalian gelatin. *Food Hydrocolloids, 18* (2), 203-213.

Hayakawa, K., Kawaguchi, M., & Kato, T. (1997). Protective colloidal effects of hydroxypropyl methyl cellulose on the stability of silicone oil emulsions. *Langmuir, 13* (23), 6069-6073.

Heertje, I., & Leunis, M. (1997). Measurement of shape and size of fat crystals by electron microscopy. *Food Science and Technology-Lebensmittel-Wissenschaft & Technologie, 30* (2), 141-146.

Heertje, I., Leunis, M., Vanzeyl, W. J. M., & Berends, E. (1987). Product Morphology of Fatty Products. *Food Microstructure, 6* (1), 1-8.

Herrington, T. M., & Sahi, S. S. (1988). Phase-Behavior of Some Sucrose Surfactants with Water and N-Decane. *Journal of the American Oil Chemists Society, 65* (10), 1677-1681.

Himawan, C., Starov, V. M., & Stapley, A. G. F. (2006). Thermodynamic and kinetic aspects of fat crystallization. *Advances in Colloid and Interface Science, 122* (1-3), 3-33.

Hoffmann, S., Koos, E., & Willenbacher, N. (2014). Using capillary bridges to tune stability and flow behavior of food suspensions. *Food Hydrocolloids, 40*, 44-52.

Hughes, N. E., Marangoni, A. G., Wright, A. J., Rogers, M. A., & Rush, J. W. E. (2009). Potential food applications of edible oil organogels. *Trends in Food Science & Technology, 20* (10), 470-480.

Hyde, S. T. (2001). Identification of lyotropic liquid crystalline mesophases. In K. Holmberg (Ed.), *Handbook of Applied Surface and Colloid Chemistry* (pp. 299-332). New York: John Wiley and Sons.

Iwanaga, K., Kawai, M., Miyazaki, M., & Kakemi, M. (2012). Application of organogels as oral controlled release formulations of hydrophilic drugs. *International Journal of Pharmaceutics, 436* (1-2), 869-872.

Jana, S., & Martini, S. (2014). Effect of High-Intensity Ultrasound and Cooling Rate on the Crystallization Behavior of Beeswax in Edible Oils. *Journal of Agricultural and Food Chemistry, 62* (41), 10192-10202.

Jana, S., & Martini, S. (2016). Physical characterization of crystalline networks formed by binary blends of waxes in soybean oil. *Food Research International, 89*, 245-253.

Jandacek, R. J., & Webb, M. R. (1978). Physical-Properties of Pure Sucrose Octaesters. *Chemistry and Physics of Lipids, 22* (2), 163-176.

Jang, A., Bae, W., Hwang, H. S., Lee, H. G., & Lee, S. (2015). Evaluation of canola oil oleogels with candelilla wax as an alternative to shortening in baked goods. *Food Chemistry, 187*, 525-529.

John, G., Zhu, G. Y., Li, J., & Dordick, J. S. (2006). Enzymatically derived sugar-containing self-assembled organogels with nanostructured morphologies. *Angewandte Chemie-International Edition, 45* (29), 4772-4775.

Kamal, M. A., & Raghunathan, V. A. (2012). Phase behavior of phospholipid-phytosterol membranes. *Soft Matter, 8* (34), 8952-8958.

Kato, T., Mizoshita, N., & Kishimoto, K. (2006). Functional liquid-crystalline assemblies: Self-organized soft materials. *Angewandte Chemie-International Edition, 45* (1), 38-68.

Kawaguchi, T., Hamanaka, T., Kito, Y., & Machida, H. (1991). Structural Studies of a Homologous Series of Alkyl Sucrose Ester Micelle by X-Ray-Scattering. *Journal of Physical Chemistry, 95* (9), 3837-3846.

Kesselman, E., & Shimoni, E. (2007). Imaging of oil/monoglyceride networks by polarizing near-field scanning optical microscopy. *Food Biophysics, 2* (2-3), 117-123.

Kim, G. Y., & Marangoni, A. G. (2017). Crystallization Behavior of High Behenic Acid Stabilizers in Liquid Oil. *Journal of the American Oil Chemists' Society, 94* (9), 1165-1173.

Kim, J., & Vanapalli, S. A. (2013). Microfluidic Production of Spherical and Nonspherical Fat Particles by Thermal Quenching of Crystallizable Oils. *Langmuir, 29* (39), 12307-12316.

Kim, J. Y., Lim, J., Lee, J., Hwang, H. S., & Lee, S. (2017). Utilization of Oleogels as a Replacement for Solid Fat in Aerated Baked Goods: Physicochemical, Rheological, and Tomographic Characterization. *Journal of Food Science, 82* (2), 445-452.

Kirilov, P., Rum, S., Gilbert, E., Roussel, L., Salmon, D., Abdayem, R., Serre, C., Villa, C., Haftek, M., Falson, F., & Pirot, F. (2014). Aqueous dispersions of organogel nanoparticles - potential systems for cosmetic and dermo-cosmetic applications. *International Journal of Cosmetic Science, 36* (4), 336-346.

Kobayashi, K., Huang, C. I., & Lodge, T. P. (1999). Thermoreversible gelation of aqueous methylcellulose solutions. *Macromolecules, 32* (21), 7070-7077.
Koos, E. (2014). Capillary suspensions: Particle networks formed through the capillary force. *Current Opinion in Colloid & Interface Science, 19* (6), 575-584.
Koos, E., Kannowade, W., & Willenbacher, N. (2014). Restructuring and aging in a capillary suspension. *Rheologica Acta, 53* (12), 947-957.
Kouzounis, D., Lazaridou, A., & Katsanidis, E. (2017). Partial replacement of animal fat by oleogels structured with monoglycerides and phytosterols in frankfurter sausages. *Meat Science, 130*, 38-46.
Kuwahara, T., Nagase, H., Endo, T., Ueda, H., & Nakagaki, M. (1996). Crystal structure of DL-12-hydroxystearic acid. *Chemistry Letters* (6), 435-436.
Kwon, S. Y., & Kim, M. W. (2001). Structure and growth control of a nonionic surfactant micelle by adding a phospholipid. *Langmuir, 17* (26), 8016-8023.
Laaman, T. R. (2011). *Hydrocolloids: fifteen practical tips*. United states: Blackwell
Lan, Y., Corradini, M. G., Weiss, R. G., Raghavan, S. R., & Rogers, M. A. (2015). To gel or not to gel: correlating molecular gelation with solvent parameters. *Chemical Society Reviews, 44* (17), 6035-6058.
Lan, Y. Q., & Rogers, M. A. (2015). 12-Hydroxystearic acid SAFiNs in aliphatic diols - a molecular oddity. *Crystengcomm, 17* (42), 8031-8038.
Larsson, K., Gabrielsson, K., & Lundberg, B. (1978). Phase Behavior of Some Aqueous Systems Involving Monoglycerides, Cholesterol and Bile-Acids. *Journal of the Science of Food and Agriculture, 29* (10), 909-914.
Lavigne, F., Bourgaux, C., & Ollivon, M. (1993). Phase-Transitions of Saturated Triglycerides. *Journal De Physique Iv, 3* (C8), 137-140.
Lawrence, G. D. (2013). Dietary Fats and Health: Dietary Recommendations in the Context of Scientific Evidence. *Advances in Nutrition, 4* (3), 294-302.
Lazzari, S., Nicoud, L., Jaquet, B., Lattuada, M., & Morbidelli, M. (2016). Fractal-like structures in colloid science. *Advances in Colloid and Interface Science, 235*, 1-13.
Lemaitre, R. N., King, I. B., Mozaffarian, D., Sootodehnia, N., & Siscovick, D. S. (2006). Trans-fatty acids and sudden cardiac death. *Atherosclerosis Supplements, 7* (2), 13-15.
Leser, M. E., Sagalowicz, L., Michel, M., & Watzke, H. J. (2006). Self-assembly of polar food lipids. *Advances in Colloid and Interface Science, 123*, 125-136.
Letourneau, J. J., Vigneau, S., Gonus, P., & Fages, J. (2005). Micronized cocoa butter particles produced by a supercritical process. *Chemical Engineering and Processing, 44* (2), 201-207.
Lin, Y., & Wagner, G. J. (1994). Rapid and Simple Method for Estimation of Sugar Esters. *Journal of Agricultural and Food Chemistry, 42* (8), 1709-1712.
Litwinenko, J. W., Rojas, A. M., Gerschenson, L. N., & Marangoni, A. G. (2002). Relationship between crystallization behavior, microstructure, and mechanical properties in a palm oil-based shortening. *Journal of the American Oil Chemists Society, 79* (7), 647-654.
Liu, C. W., Su, M., Li, X. L., Xue, T., Liu, N., Yin, J., Zhu, Y. Y., & Wu, Z. Q. (2015). Multi-stimuli-responsive chiral organogels based on peptide derivatives. *Soft Matter, 11* (28), 5727-5737.
Liu, Z. W., Han, Z., Zeng, X. A., Sun, D. W., & Aadil, R. M. (2016). Effects of vesicle components on the electro-permeability of lipid bilayers of vesicles induced by pulsed electric fields (PEF) treatment. *Journal of Food Engineering, 179*, 88-97.
Lopez-Martinez, A., Charo-Alonso, M. A., Marangoni, A. G., & Toro-Vazquez, J. F. (2015). Monoglyceride organogels developed in vegetable oil with and without ethylcellulose. *Food Research International, 72*, 37-46.
Lopez-Martinez, A., Morales-Rueda, J. A., Dibildox-Alvarado, E., Charo-Alonso, M. A., Marangoni, A. G., & Toro-Vazquez, J. F. (2014). Comparing the crystallization and rheological behavior of organogels developed by pure and commercial monoglycerides in vegetable oil. *Food Research International, 64*, 946-957.

Lopez-Rubio, A., & Gilbert, E. P. (2009). Neutron scattering: a natural tool for food science and technology research. *Trends in Food Science & Technology, 20* (11-12), 576-586.
Lupi, F. R., Gabriele, D., Baldino, N., Mijovic, P., Parisi, O. I., & Puoci, F. (2013). Olive oil/policosanol organogels for nutraceutical and drug delivery purposes. *Food & Function, 4* (10), 1512-1520.
Lupi, F. R., Gabriele, D., Baldino, N., Seta, L., de Cindio, B., & De Rose, C. (2012). Stabilization of meat suspensions by organogelation: A rheological approach. *European Journal of Lipid Science and Technology, 114* (12), 1381-1389.
Lupi, F. R., Gentile, L., Gabriele, D., Mazzulla, S., Baldino, N., & de Cindio, B. (2015). Olive oil and hyperthermal water bigels for cosmetic uses. *Journal of Colloid and Interface Science, 459*, 70-78.
Lupi, F. R., Greco, V., Baldino, N., de Cindio, B., Fischer, P., & Gabriele, D. (2016). The effects of intermolecular interactions on the physical properties of organogels in edible oils. *Journal of Colloid and Interface Science, 483*, 154-164.
Lupi, F. R., Shakeel, A., Greco, V., Rossi, C. O., Baldino, N., & Gabriele, D. (2016). A rheological and microstructural characterisation of bigels for Cosmetic and pharmaceutical uses. *Materials Science & Engineering C-Materials for Biological Applications, 69*, 358-365.
Lutz, R., Aserin, A., & Garti, N. (2005). Maillard reaction between leucine and glucose in O/W microemulsion media in comparison to aqueous solution. *Journal of Dispersion Science and Technology, 26* (5), 535-547.
Mackellar, A. J., Buckton, G., Newton, J. M., & Orr, C. A. (1994). The Controlled Crystallization of a Model Powder .2. Investigation into the Mechanism of Action of Poloxamers in Changing Crystal Properties. *International Journal of Pharmaceutics, 112* (1), 79-85.
Maleky, F., Acevedo, N. C., & Marangoni, A. G. (2012). Cooling rate and dilution affect the nanostructure and microstructure differently in model fats. *European Journal of Lipid Science and Technology, 114* (7), 748-759.
Mannock, D. A., Benesch, M. G. K., Lewis, R. N. A. H., & McElhaney, R. N. (2015). A comparative calorimetric and spectroscopic study of the effects of cholesterol and of the plant sterols beta-sitosterol and stigmasterol on the thermotropic phase behavior and organization of dipalmitoylphosphatidylcholine bilayer membranes. *Biochimica Et Biophysica Acta-Biomembranes, 1848* (8), 1629-1638.
Mannock, D. A., Lewis, R. N. A. H., & McElhaney, R. N. (2006). Comparative calorimetric and spectroscopic studies of the effects of lanosterol and cholesterol on the thermotropic phase behavior and organization of dipalmitoylphosphatidylcholine bilayer membranes. *Biophysical Journal, 91* (9), 3327-3340.
Manzocco, L., Valoppi, F., Calligaris, S., Andreatta, F., Spilimbergo, S., & Nicoli, M. C. (2017). Exploitation of κ-carrageenan aerogels as template for edible oleogel preparation. *Food Hydrocolloids, 71* (71), 68-75.
Marangoni, A. G., Acevedo, N., Maleky, F., Co, E., Peyronel, F., Mazzanti, G., Quinn, B., & Pink, D. (2012). Structure and functionality of edible fats. *Soft Matter, 8* (5), 1275-1300.
Marangoni, A. G., & Pink, D. (2015). *Edible nanostructures : a bottom-up approach*. Cambridge: Royal society of chemistry.
Marangoni, A. G., & Rousseau, D. (1996). Is plastic fat rheology governed by the fractal nature of the fat crystal network? *Journal of the American Oil Chemists Society, 73* (8), 991-994.
Martini, S., Puppo, M. C., Hartel, R. W., & Herrera, M. L. (2002). Effect of sucrose esters and sunflower oil addition on crystalline microstructure of a high-melting milk fat fraction. *Journal of Food Science, 67* (9), 3412-3418.
Martini, S., Tan, C. Y., & Jana, S. (2015). Physical Characterization of Wax/Oil Crystalline Networks. *Journal of Food Science, 80* (5), C989-C997.
Mathers, C. D., & Loncar, D. (2006). Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med, 3* (11), e442.
Matheson, A. B., Koutsos, V., Dalkas, G., Euston, S., & Clegg, P. (2017). Microstructure of beta-Sitosterol:gamma-Oryzanol Edible Organogels. *Langmuir, 33* (18), 4537-4542.

Mattice, K. D., & Marangoni, A. G. (2017). Matrix effects on the crystallization behaviour of butter and roll-in shortening in laminated bakery products. *Food Research International, 96*, 54-63.
Mattice, K. D., & Marangoni, A. G. (2018). Gelatinized wheat starch influences crystallization behaviour and structure of roll-in shortenings in laminated bakery products. *Food Chemistry, 243*, 396-402.
McClements, D. J. (2004). Protein-stabilized emulsions. *Current Opinion in Colloid & Interface Science, 9* (5), 305-313.
McClements, D. J. (2005). *Food emulsions: Principles, practice and techniques*. Boca Raton Florida: CRC Press.
McClements, D. J. (2008). Delivery of functionality in complex food systems: Physically inspired approaches from nanoscale to microscale. *Food Biophysics, 3* (2), 111-112.
McClements, D. J., Decker, E. A., Park, Y., & Weiss, J. (2008). Designing food structure to control stability, digestion, release and absorption of lipophilic food components. *Food Biophysics, 3* (2), 219-228.
Mcmullen, T. P. W., Lewis, R. N. A. H., & Mcelhaney, R. N. (1994). Comparative Differential Scanning Calorimetric and Ftir and P-31-Nmr Spectroscopic Studies of the Effects of Cholesterol and Androstenol on the Thermotropic Phase-Behavior and Organization of Phosphatidylcholine Bilayers. *Biophysical Journal, 66* (3), 741-752.
Mehta, S. B., Shribak, M., & Oldenbourg, R. (2013). Polarized light imaging of birefringence and diattenuation at high resolution and high sensitivity. *Journal of Optics, 15* (9).
Mellema, M. (2003). Mechanism and reduction of fat uptake in deep-fat fried foods. *Trends in Food Science & Technology, 14* (9), 364-373.
Meng, Z., Yang, L. J., Geng, W. X., Yao, Y. B., Wang, X. G., & Liu, Y. F. (2014). Kinetic Study on the Isothermal and Nonisothermal Crystallization of Monoglyceride Organogels. *Scientific World Journal*.
Mert, B., & Demirkesen, I. (2016a). Evaluation of highly unsaturated oleogels as shortening replacer in a short dough product. *Lwt-Food Science and Technology, 68*, 477-484.
Mert, B., & Demirkesen, I. (2016b). Reducing saturated fat with oleogel/shortening blends in a baked product. *Food Chemistry, 199*, 809-816.
Mezger, T. G. (2011). *The rheology handbook: For users of rotational and oscillatory rheometers*. Hannover, Germany: Vincentz Network GmbH & Co.
Mezzenga, R. (2007). Equilibrium and non-equilibrium structures in complex food systems. *Food Hydrocolloids, 21* (5-6), 674-682.
Mezzenga, R. (2009). *Self-assembled food mesophases, in Research Worlds-Focus on Food*: Alimentarium Foundation Editions.
Mezzenga, R., Schurtenberger, P., Burbidge, A., & Michel, M. (2005). Understanding foods as soft materials. *Nature Materials, 4* (10), 729-740.
Micha, R., Khatibzadeh, S., Shi, P. L., Fahimi, S., Lim, S., Andrews, K. G., Engell, R. E., Powles, J., Ezzati, M., Mozaffarian, D., & Dis, G. B. D. N. C. (2014). Global, regional, and national consumption levels of dietary fats and oils in 1990 and 2010: a systematic analysis including 266 country-specific nutrition surveys. *Bmj-British Medical Journal, 348*.
Michalak, Z., Muzzio, M., Milianta, P. J., Giacomini, R., & Lee, S. (2013). Effect of Monoglyceride Structure and Cholesterol Content on Water Permeability of the Droplet Bilayer. *Langmuir, 29* (51), 15919-15925.
Mishima, S., Suzuki, A., Sato, K., & Ueno, S. (2016). Formation and Microstructures of Whipped Oils Composed of Vegetable Oils and High-Melting Fat Crystals. *Journal of the American Oil Chemists Society, 93* (11), 1453-1466.
Moens, K., De Clercq, N., Verstringe, S., & Dewettinck, K. (2015). Revealing the Influence of Tempering on Polymorphism and Crystal Arrangement in Semicrystalline Oil-in-Water Emulsions. *Crystal Growth & Design, 15* (12), 5693-5704.

Molinier, V., Kouwer, P. H. J., Fitremann, J., Bouchu, A., Mackenzie, G., Queneau, Y., & Goodby, J. W. (2006). Self-organizing properties of monosubstituted sucrose fatty acid esters: The effects of chain length and unsaturation. *Chemistry-a European Journal, 12* (13), 3547-3557.

Molinier, V., Kouwer, P. J. J., Fitremann, J., Bouchu, A., Mackenzie, G., Queneau, Y., & Goodby, J. W. (2007). Shape dependence in the formation of condensed phases exhibited by disubstituted sucrose esters. *Chemistry-a European Journal, 13* (6), 1763-1775.

Morales, M. E., Gallardo, V., Clares, B., Garcia, M. B., & Ruiz, M. A. (2009). Study and description of hydrogels and organogels as vehicles for cosmetic active ingredients. *Journal of Cosmetic Science, 60* (6), 627-636.

Moreau, R. A., Kohout, K., & Singh, V. (2002). Temperature-enhanced alumina HPLC method for the analysis of wax esters, sterol esters, and methyl esters. *Lipids, 37* (12), 1201-1204.

Moreno-Calvo, E., Temelli, F., Cordoba, A., Masciocchi, N., Veciana, J., & Ventosa, N. (2014). A New Microcrystalline Phytosterol Polymorph Generated Using CO2-Expanded Solvents. *Crystal Growth & Design, 14* (1), 58-68.

Mozaffarian, D., Katan, M. B., Ascherio, A., Stampfer, M. J., & Willett, W. C. (2006). Medical progress - Trans fatty acids and cardiovascular disease. *New England Journal of Medicine, 354* (15), 1601-1613.

Munuklu, P., & Jansens, P. J. (2007). Particle formation of an edible fat (rapeseed 70) using the supercritical melt micronization (ScMM) process. *Journal of Supercritical Fluids, 40* (3), 433-442.

Mutanen, M., & Aro, A. (1997). Coagulation and fibrinolysis factors in healthy subjects consuming high stearic or trans fatty acid diets. *Thrombosis and Haemostasis, 77* (1), 99-104.

Mykhaylyk, O. O., & Hamley, I. W. (2004). The packing of Triacylglycerols from SAXS measurements: Application to the structure of 1,3-distearoyl-2-oleoyl-sn-glycerol crystal phases. *Journal of Physical Chemistry B, 108* (23), 8069-8083.

Nakano, A., & Masaki, N. (1989). Migration inhibitor for fats and oilf for baked cakes and process for producing composite baked cakes with the use of the same. In U. Patent (Ed.), (Vol. 4,865 162). United State of America: US Patent.

Narine, S. S., & Marangoni, A. G. (1999). Relating structure of fat crystal networks to mechanical properties: a review. *Food Research International, 32* (4), 227-248.

Nasatto, P. L., Pignon, F., Silveira, J. L. M., Duarte, M. E. R., Noseda, M. D., & Rinaudo, M. (2015). Methylcellulose, a Cellulose Derivative with Original Physical Properties and Extended Applications. *Polymers, 7* (5), 777-803.

Nikiforidis, C. V. (2015). Lipid Mesophase Nanostructures. In A. G. Marangoni & D. Pink (Eds.), *Edible Nanostructures* (pp. 114-143). United Kingdom: Royal Society of Chemistry.

Nikiforidis, C. V., Gilbert, E. P., & Scholten, E. (2015). Organogel formation via supramolecular assembly of oleic acid and sodium oleate. *Rsc Advances, 5* (59), 47466-47475.

Nikiforidis, C. V., & Scholten, E. (2014). Self-assemblies of lecithin and alpha-tocopherol as gelators of lipid material. *Rsc Advances, 4* (5), 2466-2473.

Norton, J. E., Espinosa, Y. G., Watson, R. L., Spyropoulos, F., & Norton, I. T. (2015). Functional food microstructures for macronutrient release and delivery. *Food & Function, 6* (3), 663-678.

O'Sullivan, C., Acevedo, N., Peyronel, F., & Marangoni, A. G. (2015). Fat Nanostructure. In A. G. Marangoni & D. Pink (Eds.), *Edible Nanostructures* (pp. 6-40). United Kingdom: Royal Society of Chemistry.

O'Sullivan, C. M., Barbut, S., & Marangoni, A. G. (2016). Edible oleogels for the oral delivery of lipid soluble molecules: Composition and structural design considerations. *Trends in Food Science & Technology, 57*, 59-73.

O'Sullivan, C. M., Davidovich-Pinhas, M., Wright, A. J., Barbut, S., & Marangoni, A. G. (2017). Ethylcellulose oleogels for lipophilic bioactive delivery - effect of oleogelation on in vitro bioaccessibility and stability of beta-carotene. *Food & Function, 8* (4), 1438-1451.

Ojijo, N. K., Neeman, I., Eger, S., & Shimoni, E. (2004). Effects of monoglyceride content, cooling rate and shear on the rheological properties of olive oil/monoglyceride gel networks. *Journal of the Science of Food and Agriculture, 84* (12), 1585-1593.
Patel, A. R. (2015). *Alternative Routes to Oil Structuring*.
Patel, A. R. (2017). A colloidal gel perspective for understanding oleogelation. *Current Opinion in Food Science, 15*, 1-7.
Patel, A. R. (2017). Methylcellulose-coated microcapsules of Palm stearine as structuring templates for creating hybrid oleogels. *Materials Chemistry and Physics, 195*, 268-274.
Patel, A. R., Babaahmadi, M., Lesaffer, A., & Dewettinck, K. (2015). Rheological Profiling of Organogels Prepared at Critical Gelling Concentrations of Natural Waxes in a Triacylglycerol Solvent. *Journal of Agricultural and Food Chemistry, 63* (19), 4862-4869.
Patel, A. R., Cludts, N., Bin Sintang, M. D., Lesaffer, A., & Dewettinck, K. (2014). Edible oleogels based on water soluble food polymers: preparation, characterization and potential application. *Food & Function, 5* (11), 2833-2841.
Patel, A. R., Cludts, N., Bin Sintang, M. D., Lewille, B., Lesaffer, A., & Dewettinck, K. (2014). Polysaccharide-Based Oleogels Prepared with an Emulsion-Templated Approach. *Chemphyschem, 15* (16), 3435-3439.
Patel, A. R., & Dewettinck, K. (2015). Current update on the influence of minor lipid components, shear and presence of interfaces on fat crystallization. *Current Opinion in Food Science, 3*, 65-70.
Patel, A. R., & Dewettinck, K. (2016). Edible oil structuring: an overview and recent updates. *Food & Function, 7* (1), 20-29.
Patel, A. R., Mankoc, B., Bin Sintang, M. D., Lesaffer, A., & Dewettinck, K. (2015). Fumed silica-based organogels and 'aqueous-organic' bigels. *Rsc Advances, 5* (13), 9703-9708.
Patel, A. R., Rajarethinem, P. S., Cludts, N., Lewille, B., De Vos, W. H., Lesaffer, A., & Dewettinck, K. (2015). Biopolymer-Based Structuring of Liquid Oil into Soft Solids and Oleogels Using Water-Continuous Emulsions as Templates. *Langmuir, 31* (7), 2065-2073.
Patel, A. R., Rajarethinem, P. S., Gredowska, A., Turhan, O., Lesaffer, A., De Vos, W. H., Van de Walle, D., & Dewettinck, K. (2014). Edible applications of shellac oleogels: spreads, chocolate paste and cakes. *Food & Function, 5* (4), 645-652.
Patel, A. R., Rodriguez, Y., Lesaffer, A., & Dewettinck, K. (2014). High internal phase emulsion gels (HIPE-gels) prepared using food-grade components. *Rsc Advances, 4* (35), 18136-18140.
Patel, A. R., Schatteman, D., De Vos, W. H., Lesaffer, A., & Dewettinck, K. (2013). Preparation and rheological characterization of shellac oleogels and oleogel-based emulsions. *Journal of Colloid and Interface Science, 411*, 114-121.
Patel, A. R., Schatteman, D., Lesaffer, A., & Dewettinck, K. (2013). A foam-templated approach for fabricating organogels using a water-soluble polymer. *Rsc Advances, 3* (45), 22900-22903.
Perez, O. E., Carrera-Sanchez, C., Rodriguez-Patino, J. M., & Pilosof, A. M. R. (2007). Adsorption dynamics and surface activity at equilibrium of whey proteins and hydroxypropyl-methyl-cellulose mixtures at the air-water interface. *Food Hydrocolloids, 21* (5-6), 794-803.
Perlman, D. (2013). Liquid crystalline phytosterol-glycerine complex for enhanced bioavailability and water dispersal. In U. S. Patent (Ed.), (Vol. US 8,460,738 B1). United States: Brandeis University.
Pernetti, M., van Malssen, K., Kalnin, D., & Floter, E. (2007). Structuring edible oil with lecithin and sorbitan tri-stearate. *Food Hydrocolloids, 21* (5-6), 855-861.
Pernetti, M., van Malssen, K. F., Floter, E., & Bot, A. (2007). Structuring of edible oils by alternatives to crystalline fat. *Current Opinion in Colloid & Interface Science, 12* (4-5), 221-231.
Peyronel, F., Campos, R., & Marangoni, A. G. (2016). Prevention of oil migration in palm mid fraction and palm olein using a stabilizer rich in behenic acid. *Food Research International, 88*, 52-60.
Peyronel, F., Pink, D. A., & Marangoni, A. G. (2014). Triglyceride nanocrystal aggregation into polycrystalline colloidal networks: Ultra-small angle X-ray scattering, models and computer simulation. *Current Opinion in Colloid & Interface Science, 19* (5), 459-470.

Peyronel, F., Quinn, B., Marangoni, A. G., & Pink, D. A. (2014). Ultra small angle x-ray scattering in complex mixtures of triacylglycerols. *Journal of Physics-Condensed Matter, 26* (46).

Puppo, M. C., Martini, S., Hartel, R. W., & Herrera, M. L. (2002). Effects of sucrose esters on isothermal crystallization and rheological Behavior of blends of milk-fat fraction sunflower oil. *Journal of Food Science, 67* (9), 3419-3426.

Raghavan, S. R., & Douglas, J. F. (2012). The conundrum of gel formation by molecular nanofibers, wormlike micelles, and filamentous proteins: gelation without cross-links? *Soft Matter, 8* (33), 8539-8546.

Ramel, P. R., Co, E. D., Acevedo, N. C., & Marangoni, A. G. (2016). Structure and functionality of nanostructured triacylglycerol crystal networks. *Progress in Lipid Research, 64*, 231-242.

Rao, M. A. (2007). Influence of food microstructure on food rheology. In D. J. McClements (Ed.), *Understanding and controlling the microstructure of complex foods* (pp. 411-424). United Kingdom: Woodhead Publishing in Food Science, Technology and Nutrition.

Raut, S., Bhadoriya, S. S., Uplanchiwar, V., Mishra, V., Gahane, A., & Jain, S. K. (2012). Lecithin organogel: A unique micellar system for the delivery of bioactive agents in the treatment of skin aging. *Acta Pharmaceutica Sinica B, 2* (1), 8-15.

Rhim, J. W., & Ng, P. K. W. (2007). Natural biopolymer-based nanocomposite films for packaging applications. *Critical Reviews in Food Science and Nutrition, 47* (4), 411-433.

Ribeiro, H. S., Gupta, R., Smith, K. W., van Malssen, K. F., Popp, A. K., & Velikov, K. P. (2016). Super-cooled and amorphous lipid-based colloidal dispersions for the delivery of phytosterols. *Soft Matter, 12* (27), 5835-5846.

Rodriguez-Abreu, C., Aramaki, K., Tanaka, Y., Lopez-Quintela, M. A., Ishitobi, M., & Kunieda, H. (2005). Wormlike micelles and microemulsions in aqueous mixtures of sucrose esters and nonionic cosurfactants. *Journal of Colloid and Interface Science, 291* (2), 560-569.

Rodriguez, C., Acharya, D. P., Hinata, S., Ishitobi, M., & Kunieda, H. (2003). Effect of ionic surfactants on the phase behavior and structure of sucrose ester/water/oil systems. *Journal of Colloid and Interface Science, 262* (2), 500-505.

Rogers, M. A. (2009). Novel structuring strategies for unsaturated fats - Meeting the zero-trans, zero-saturated fat challenge: A review. *Food Research International, 42* (7), 747-753.

Rogers, M. A., Strober, T., Bot, A., Toro-Vazquez, J. F., Stortz, T., & Marangoni, A. G. (2014). Edible oleogels in molecular gastronomy. *International journal of gastronomy and food science, 2* (1), 22-31.

Rogers, M. A., Wright, A. J., & Marangoni, A. G. (2005). Microstructure of fat crystallizing on a collagenous surface. *European Journal of Lipid Science and Technology, 107* (9), 684-688.

Rogers, M. A., Wright, A. J., & Marangoni, A. G. (2008). Engineering the oil binding capacity and crystallinity of self-assembled fibrillar networks of 12-hydroxystearic acid in edible oils. *Soft Matter, 4* (7), 1483-1490.

Romoscanu, A. I., & Mezzenga, R. (2006). Emulsion-templated fully reversible protein-in-oil gels. *Langmuir, 22* (18), 7812-7818.

Rosenbau.G, Holmes, K. C., & Witz, J. (1971). Synchrotron Radiation as a Source for X-Ray Diffraction. *Nature, 230* (5294), 434-&.

Rossi, L., Sacanna, S., & Velikov, K. P. (2011). Cholesteric colloidal liquid crystals from phytosterol rod-like particles. *Soft Matter, 7* (1), 64-67.

Rossi, L., ten Hoorn, J. W. M. S., Melnikov, S. M., & Velikov, K. P. (2010). Colloidal phytosterols: synthesis, characterization and bioaccessibility. *Soft Matter, 6* (5), 928-936.

Sagalowicz, L., Leser, M. E., Watzke, H. J., & Michel, M. (2006). Monoglyceride self-assembly structures as delivery vehicles. *Trends in Food Science & Technology, 17* (5), 204-214.

Sagalowicz, L., Mezzenga, R., & Leser, M. E. (2006). Investigating reversed liquid crystalline mesophases. *Current Opinion in Colloid & Interface Science, 11* (4), 224-229.

Sagalowicz, L., Michel, M., Blank, I., Schafer, O., & Leser, M. E. (2017). Self-assembly in food - A concept for structure formation inspired by Nature. *Current Opinion in Colloid & Interface Science, 28* (28), 87-95.

Sahoo, S., Kumar, N., Bhattacharya, C., Sagiri, S. S., Jain, K., Pal, K., Ray, S. S., & Nayak, B. (2011). Organogels: Properties and Applications in Drug Delivery. *Designed Monomers and Polymers, 14* (2), 95-108.

Salo, P., & Wester, I. (2005). Low-fat formulations of plant stanols and sterols. *Am J Cardiol, 96* (1A), 51D-54D.

Sameiro, M., & Goncalves, T. (2009). Fluorescent Labeling of Biomolecules with Organic Probes. *Chemical Reviews, 109* (1), 190-212.

Sandoval, C., Ortega, A., Sanchez, S. A., Morales, J., & Gunther, G. (2015). Structuration in the Interface of Direct and Reversed Micelles of Sucrose Esters, Studied by Fluorescent Techniques. *PLoS One, 10* (4).

Sandra, S., Decker, E. A., & McClements, D. J. (2008). Effect of interfacial protein cross-linking on the in vitro digestibility of emulsified corn oil by pancreatic lipase. *Journal of Agricultural and Food Chemistry, 56* (16), 7488-7494.

Sangeetha, N. M., & Maitra, U. (2005). Supramolecular gels: Functions and uses. *Chemical Society Reviews, 34* (10), 821-836.

Sarkar, N. (1995). Kinetics of Thermal Gelation of Methylcellulose and Hydroxypropylmethylcellulose in Aqueous-Solutions. *Carbohydrate Polymers, 26* (3), 195-203.

Sato, K., Bayes-Garcia, L., Calvet, T., Cuevas-Diarte, M. A., & Ueno, S. (2013). External factors affecting polymorphic crystallization of lipids. *European Journal of Lipid Science and Technology, 115* (11), 1224-1238.

Sato, K., & Ueno, S. (2011). Crystallization, transformation and microstructures of polymorphic fats in colloidal dispersion states. *Current Opinion in Colloid & Interface Science, 16* (5), 384-390.

Sawalha, H., den Adel, R., Venema, P., Bot, A., Floter, E., & van der Linden, E. (2012). Organogel-Emulsions with Mixtures of beta-Sitosterol and gamma-Oryzanol: Influence of Water Activity and Type of Oil Phase on Gelling Capability. *Journal of Agricultural and Food Chemistry, 60* (13), 3462-3470.

Scalia, S., Young, P. M., & Traini, D. (2015). Solid lipid microparticles as an approach to drug delivery. *Expert Opin Drug Deliv, 12* (4), 583-599.

Scartazzini, R., & Luisi, P. L. (1988). Organogels from lecithins. *The Journal of Physical Chemistry, 92* (3), 829-833.

Schaink, H. M., van Malssen, K. F., Morgado-Alves, S., Kalnin, D., & van der Linden, E. (2007). Crystal network for edible oil organogels: Possibilities and limitations of the fatty acid and fatty alcohol systems. *Food Research International, 40* (9), 1185-1193.

Shaikh, I. M., Jadhav, K. R., Gide, P. S., Kadam, V. J., & Pisal, S. S. (2006). Topical delivery of aceclofenac from lecithin organogels: preformulation study. *Curr Drug Deliv, 3* (4), 417-427.

Shaikh, S. R., Cherezov, V., Caffrey, M., Soni, S. P., LoCascio, D., Stillwell, W., & Wassall, S. R. (2006). Molecular organization of cholesterol in unsaturated phosphatidylethanolamines: X-ray diffraction and solid state H-2 NMR reveal differences with phosphatidylcholines. *Journal of the American Chemical Society, 128* (16), 5375-5383.

Sharma, S. C., Acharya, D. P., & Aramaki, K. (2007). Viscoelastic micellar solutions in a mixed nonionic fluorinated surfactants system and the effect of oils. *Langmuir, 23* (10), 5324-5330.

Sharma, S. C., Rodriguez-Abreu, C., Shrestha, L. K., & Aramaki, K. (2007a). Oil-induced anomalous thermoresponsive viscoelasticity in fluorinated surfactant systems. *Journal of Physical Chemistry B, 111* (42), 12146-12153.

Sharma, S. C., Rodriguez-Abreu, C., Shrestha, L. K., & Aramaki, K. (2007b). Short haired wormlike micelles in mixed nonionic fluorocarbon surfactants. *Journal of Colloid and Interface Science, 314* (1), 223-229.

Shchipunov, Y. A. (2001). Lecithin organogel - A micellar system with unique properties. *Colloids and Surfaces a-Physicochemical and Engineering Aspects, 183*, 541-554.

Shchipunov, Y. A., & Shumilina, E. V. (1995). Lecithin Bridging by Hydrogen-Bonds in the Organogel. *Materials Science & Engineering C-Biomimetic Materials Sensors and Systems, 3* (1), 43-50.

Shchipunov, Y. A., Shumilina, E. V., Ulbricht, W., & Hoffmann, H. (1999). The branching of reversed polymer-like micelles of lecithin by sugar-containing surfactants. *Journal of Colloid and Interface Science, 211* (1), 81-88.

Shih, W. H., Shih, W. Y., Kim, S. I., Liu, J., & Aksay, I. A. (1990). Scaling Behavior of the Elastic Properties of Colloidal Gels. *Physical Review A, 42* (8), 4772-4779.

Si, H. G., Cheong, L. Z., Huang, J. H., Wang, X. G., & Zhang, H. (2016). Physical Properties of Soybean Oleogels and Oil Migration Evaluation in Model Praline System. *Journal of the American Oil Chemists Society, 93* (8), 1075-1084.

Singh, A., Auzanneau, F. I., & Rogers, M. A. (2017). Advances in edible oleogel technologies - A decade in review. *Food Research International, 97*, 307-317.

Smith, A. K. (2015). Imaging nanostructure. In A. G. Marangoni & D. Pink (Eds.), *Edible Nanostructures* (pp. 210-229). United Kingdom: The Royal Society of Chemistry.

Smith, E. A., Wang, W., & Dea, P. K. (2012). Effects of cholesterol on phospholipid membranes: inhibition of the interdigitated gel phase of F-DPPC and F-DPPC/DPPC. *Chemistry and Physics of Lipids, 165* (2), 151-159.

Smith, K. W., Bhaggan, K., Talbot, G., & van Malssen, K. F. (2011). Crystallization of Fats: Influence of Minor Components and Additives. *Journal of the American Oil Chemists Society, 88* (8), 1085-1101.

Stokes, J. R., Davies, G. A., Macakova, L., Yakubov, G., Bongaerts, J., & Rossetti, D. (2008). From rheology to tribology: Multiscale dynamics of biofluids, food emulsions and soft matter. *Xvth International Congress on Rheology - the Society of Rheology 80th Annual Meeting, Pts 1 and 2, 1027*, 1171-1173.

Stokes, J. R., & Frith, W. J. (2008). Rheology of gelling and yielding soft matter systems. *Soft Matter, 4* (6), 1133-1140.

Stubenrauch, C. (2001). Sugar surfactants - aggregation, interfacial, and adsorption phenomena. *Current Opinion in Colloid & Interface Science, 6* (2), 160-170.

Szuts, A., Budai-Szucs, M., Eros, I., Otomo, N., & Szabo-Revesz, P. (2010). Study of gel-forming properties of sucrose esters for thermosensitive drug delivery systems. *International Journal of Pharmaceutics, 383* (1-2), 132-137.

Szuts, A., Pallagi, E., Regdon, G., Aigner, Z., & Szabo-Revesz, P. (2007). Study of thermal behaviour of sugar esters. *International Journal of Pharmaceutics, 336* (2), 199-207.

Tan, S. Y., Peh, E. W. Y., Marangoni, A. G., & Henry, C. J. (2017). Effects of liquid oil vs. oleogel co-ingested with a carbohydrate-rich meal on human blood triglycerides, glucose, insulin and appetite. *Food & Function, 8* (1), 241-249.

Tang, D. M., & Marangoni, A. G. (2006a). Microstructure and fractal analysis of fat crystal networks. *Journal of the American Oil Chemists Society, 83* (5), 377-388.

Tang, D. M., & Marangoni, A. G. (2006b). Quantitative study on the microstructure of colloidal fat crystal networks and fractal dimensions. *Advances in Colloid and Interface Science, 128*, 257-265.

Tantipolphan, R., Rades, T., Strachan, C. J., Gordon, K. C., & Medlicott, N. J. (2006). Analysis of lecithin-cholesterol mixtures using Raman spectroscopy. *Journal of Pharmaceutical and Biomedical Analysis, 41* (2), 476-484.

Tavernier, I., Doan, C. D., Van de Walle, D., Danthine, S., Rimaux, T., & Dewettinck, K. (2017). Sequential crystallization of high and low melting waxes to improve oil structuring in wax-based oleogels. *Rsc Advances, 7* (20), 12113-12125.

Tavernier, I., Patel, A. R., Van der Meeren, P., & Dewettinck, K. (2017). Emulsion-templated liquid oil structuring with soy protein and soy protein: kappa-carrageenan complexes. *Food Hydrocolloids, 65*, 107-120.

Terech, P., & Weiss, R. G. (1997). Low molecular mass gelators of organic liquids and the properties of their gels. *Chemical Reviews, 97* (8), 3133-3159.

Toro-Vazquez, J. F., Morales-Rueda, J. A., Dibildox-Alvarado, E., Charo-Alonso, M., Alonzo-Macias, M., & Gonzalez-Chavez, M. M. (2007). Thermal and textural properties of organogels developed

by candelilla wax in safflower oil. *Journal of the American Oil Chemists Society, 84* (11), 989-1000.
Toseland, C. P. (2013). Fluorescent labeling and modification of proteins. *J Chem Biol, 6* (3), 85-95.
Tran, T., & Rousseau, D. (2016). Influence of shear on fat crystallization. *Food Research International, 81*, 157-162.
Trappe, V., & Sandkuhler, P. (2004). Colloidal gels - low-density disordered solid-like states. *Current Opinion in Colloid & Interface Science, 8* (6), 494-500.
Ubbink, J. (2012). Soft matter approaches to structured foods: from "cook-and-look" to rational food design? *Faraday Discussions, 158*, 9-35.
Ubbink, J., Burbidge, A., & Mezzenga, R. (2008). Food structure and functionality: a soft matter perspective. *Soft Matter, 4* (8), 1569-1581.
Ubeyitogullari, A., & Ciftci, O. N. (2016). Phytosterol nanoparticles with reduced crystallinity generated using nanoporous starch aerogels. *Rsc Advances, 6* (110), 108319-108327.
Ullrich, S., Metz, H., & Mader, K. (2008). Sucrose ester nanodispersions: Microviscosity and viscoelastic properties. *European Journal of Pharmaceutics and Biopharmaceutics, 70* (2), 550-555.
Vaikousi, H., Lazaridou, A., Biliaderis, C. G., & Zawistowski, J. (2007). Phase transitions, solubility, and crystallization kinetics of phytosterols and phytosterol-oil blends. *Journal of Agricultural and Food Chemistry, 55* (5), 1790-1798.
van de Velde, F., Weinbreck, F., Edelman, M. W., van der Linden, E., & Tromp, R. H. (2003). Visualisation of biopolymer mixtures using confocal scanning laser microscopy (CSLM) and covalent labelling techniques. *Colloids and Surfaces B-Biointerfaces, 31* (1-4), 159-168.
van der Sman, R. G. (2012). Soft matter approaches to food structuring. *Adv Colloid Interface Sci, 176-177* (176-177), 18-30.
van der Sman, R. G. M., & van der Goot, A. J. (2009). The science of food structuring. *Soft Matter, 5* (3), 501-510.
Vanapalli, S. A., Palanuwech, J., & Coupland, J. N. (2002a). Influence of fat crystallization on the stability of flocculated emulsions. *J Agric Food Chem, 50* (18), 5224-5228.
Vanapalli, S. A., Palanuwech, J., & Coupland, J. N. (2002b). Stability of emulsions to dispersed phase crystallization: effect of oil type, dispersed phase volume fraction, and cooling rate. *Colloids and Surfaces a-Physicochemical and Engineering Aspects, 204* (1-3), 227-237.
Vereecken, J., De Graef, V., Smith, K. W., Wouters, J., & Dewettinck, K. (2010). Effect of TAG composition on the crystallization behaviour of model fat blends with the same saturated fat content. *Food Research International, 43* (8), 2057-2067.
Vereecken, J., Meeussen, W., Foubert, I., Lesaffer, A., Wouters, J., & Dewettinck, K. (2009). Comparing the crystallization and polymorphic behaviour of saturated and unsaturated monoglycerides. *Food Research International, 42* (10), 1415-1425.
Verstringe, S., Danthine, S., Blecker, C., Depypere, F., & Dewettinck, K. (2013). Influence of monopalmitin on the isothermal crystallization mechanism of palm oil. *Food Research International, 51* (1), 344-353.
Verstringe, S., Dewettinck, K., Ueno, S., & Sato, K. (2014). Triacylglycerol Crystal Growth: Templating Effects of Partial Glycerols Studied with Synchrotron Radiation Microbeam X-ray Diffraction. *Crystal Growth & Design, 14* (10), 5219-5226.
Verstringe, S., Moens, K., De Clercq, N., & Dewettinck, K. (2015). Crystallization behavior of monoacylglycerols in a hydrophobic and a hydrophilic solvent. *Food Research International, 67*, 25-34.
Vilgis, T. A. (2015). Gels: model systems for soft matter food physics. *Current Opinion in Food Science, 3*, 71-84.
Vintiloiu, A., & Leroux, J. C. (2008). Organogels and their use in drug delivery - A review. *Journal of Controlled Release, 125* (3), 179-192.

von Bonsdorff-Nikander, A., Karjalainen, M., Rantanen, J., Christiansen, L., & Yliruusi, J. (2003). Physical stability of a microcrystalline beta-sitosterol suspension in oil. *European Journal of Pharmaceutical Sciences, 19* (4), 173-179.

vons Bonsdorff-Nikander, A., Lievonen, S., Christiansen, L., Rantanen, J., & Yliruusi, J. (2003). Physical changes of beta-sitosterol suspensions during heating. *European Journal of Pharmaceutical Sciences, 19*, S40-S40.

Wang, C., Wang, Z. Q., & Zhang, X. (2012). Amphiphilic Building Blocks for Self-Assembly: From Amphiphiles to Supra-amphiphiles. *Accounts of Chemical Research, 45* (4), 608-618.

Wang, F. C., Gravelle, A. J., Blake, A. I., & Marangoni, A. G. (2016). Novel trans fat replacement strategies. *Current Opinion in Food Science, 7*, 27-34.

Wang, J. H., Bartlett, J. D., Dunn, A. C., Small, S., Willis, S. L., Driver, M. J., & Lewis, A. L. (2005). The use of rhodamine 6G and fluorescence microscopy in the evaluation of phospholipid-based polymeric biomaterials. *Journal of Microscopy-Oxford, 217*, 216-224.

Wassell, P., Okamura, A., Young, N. W. G., Bonwick, G., Smith, C., Sato, K., & Ueno, S. (2012). Synchrotron Radiation Macrobeam and Microbeam X-ray Diffraction Studies of Interfacial Crystallization of Fats in Water-in-Oil Emulsions. *Langmuir, 28* (13), 5539-5547.

Weiss, J., Decker, E. A., McClements, D. J., Kristbergsson, K., Helgason, T., & Awad, T. (2008). Solid lipid nanoparticles as delivery systems for bioactive food components. *Food Biophysics, 3* (2), 146-154.

Weiss, R. G. (2014). The Past, Present, and Future of Molecular Gels. What Is the Status of the Field, and Where Is It Going? *Journal of the American Chemical Society, 136* (21), 7519-7530.

Whitesides, G. M., & Boncheva, M. (2002). Beyond molecules: Self-assembly of mesoscopic and macroscopic components. *Proceedings of the National Academy of Sciences of the United States of America, 99* (8), 4769-4774.

Wollgarten, S., Yuce, C., Koos, E., & Willenbacher, N. (2016). Tailoring flow behavior and texture of water based cocoa suspensions. *Food Hydrocolloids, 52*, 167-174.

Wright, A. J., & Marangoni, A. G. (2007). Time, temperature, and concentration dependence of ricinelaidic acid-canola oil organogelation. *Journal of the American Oil Chemists Society, 84* (1), 3-9.

Wu, Y. P., Wu, S., Zou, G., & Zhang, Q. J. (2011). Solvent effects on structure, photoresponse and speed of gelation of a dicholesterol-linked azobenzene organogel. *Soft Matter, 7* (19), 9177-9183.

Yaghmur, A., de Campo, L., Sagalowicz, L., Leser, M. E., & Glatter, O. (2005). Emulsified microemulsions and oil-containing liquid crystalline phases. *Langmuir, 21* (2), 569-577.

Yaghmur, A., de Campo, L., Salentinig, S., Sagalowicz, L., Leser, M. E., & Glatter, O. (2006). Oil-loaded monolinolein-based particles with confined inverse discontinuous cubic structure (Fd3m). *Langmuir, 22* (2), 517-521.

Yang, D. X., Chen, X. W., & Yang, X. Q. (2017). Phytosterols-based Oleogels Self-assembled with Monoglyceride for Controlled Volatile Release. *J Sci Food Agric*.

Yilmaz, E., & Ogutcu, M. (2015a). Oleogels as spreadable fat and butter alternatives: sensory description and consumer perception. *Rsc Advances, 5* (62), 50259-50267.

Yilmaz, E., & Ogutcu, M. (2015b). The texture, sensory properties and stability of cookies prepared with wax oleogels. *Food & Function, 6* (4), 1194-1204.

Yoshikawa, S., Kida, H., & Sato, K. (2015). Fat crystallization with talc particles is influenced by particle size, concentration, and cooling rate. *European Journal of Lipid Science and Technology, 117* (6), 858-868.

Yu, R., Lin, N. B., Yu, W. D., & Liu, X. Y. (2015). Crystal networks in supramolecular gels: formation kinetics and mesoscopic engineering principles. *Crystengcomm, 17* (42), 7986-8010.

Zhang, M., Yang, B., Liu, W., & Li, S. (2017). Influence of hydroxypropyl methylcellulose, methylcellulose, gelatin, poloxamer 407 and poloxamer 188 on the formation and stability of soybean oil-in-water emulsions. *Asian Journal of pharmaceutical sciences, 12* (6), 521-531.

Zychowski, L. M., Logan, A., Augustin, M. A., Kelly, A. L., Zabara, A., O'Mahony, J. A., Conn, C. E., & Auty, M. A. E. (2016). Effect of Phytosterols on the Crystallization Behavior of Oil-in-Water Milk Fat Emulsions. *Journal of Agricultural and Food Chemistry, 64* (34), 6546-6554.

Curriculum vitae

Mohd Dona Bin Sintang
10/12/1986, Sandakan (Malaysia)
+32487732378
MohdDonaBin.Sintang@UGent.be and donasintang@gmail.com

Education

2011-2012 The University of New South Wales (UNSW), Australia

Faculty of Chemical Engineering

- Master of Science (M.Sc) in Food Science and Technology (by Coursework)

2008-2010 University Teknologi Mara (UiTM), Malaysia

Faculty of Applied Science

- Bachelor of Science (Hons) in Food Science and Technology
- Thesis: “Microbiological quality of ready-to-eat foods”
- Promotor/Supervisor: Mdm Siti Nor Baizura Md Zain

2005-2008 University Teknologi Mara (UiTM), Malaysia

Faculty of Applied Science

- Diploma in Food Technology

Professional activities

2014- Phd Student (Malaysian Government Schorlarship)

Vandemoortele Centre Lipid Science and Technology
Laboratory of Food Technology and Engineering (FTE)
Faculty of Bioscience Engineering
Ghent University

- Phd research: Towards identification of synergistic combinations of structurants for edible liquid-oil soft matter systems
- Promotors: Prof. dr. ir. Koen Dewettinck and dr. Tom Rimaux

2013- now Assistant lecturer (Tutor) (Study leave)

Faculty of Food Science and Nutrition
Universiti Malaysia Sabah, Kota Kinabalu Malaysia

2011-2011 Health Advisor

Watson personal care, Malaysia

Publications in international peer-reviewed journals

Bin Sintang, M.D., Danthine, S., Brown, A., Van de Walle, D., Patel, A.R., Tavernier, A., Rimaux, T., & Dewettinck, K. (2017). Phytosterol-induced viscoelasticity of oleogels prepared by using monoglycerides. **Food Research International**, Article in press

Bin Sintang, M.D., Danthine, S., Rimaux, T., Patel, A.R., Van de Walle, D., & Dewettinck, K. (2017). Mixed surfactant system of sucrose esters and lecithin as a synergistic approach for oil structuring. **Journal of Colloid and Interface Science**, 504, 387-396

Bin Sintang, M.D., Rimaux, T., Van de Walle, D., Dewettinck, K., & Patel, A.R. (2017). Oil structuring properties of monoglycerides and phytosterols mixtures. **European Journal of Lipid Science and Technology**, 119(3) 1438-1452

Doan, C.D., Tavernier, I., **Bin Sintang, M,D.,** Danthine, S., Van de Walle, D., Rimaux, T., & Dewettinck, K. (2017). Crystallization and gelation behavior of low- and high melting waxes in rice bran oil: a case-study on berry wax and sunflower wax. **Food Biophysics**, 12(1), 97-108

Saputro, A.D., Van de Walle, D., Kadivar, S., **Bin Sintang, M.D.,** Van der Meeren, P., & Dewettinkc, K. (2017). Investigating the rheological, microstructural and textural properties of chocolates sweetened with palm sap-based sugar by partial replacement. **European Food Research and Technology**. https://doi.org/10.1007/s00217-017-2877-3

Patel, A.R., Mankoc, B., **Bin Sintang, M.D.,** Lesaffer, A., & Dewettinck, K. (2015). Fumed silica-based organogels and 'aqueous-organic' bigels. **RSC Advances**. 5(13), 9703-9708

Patel, A.R., Cludts, N., **Bin Sintang, M.D.**, Lewille, B., Lesaffer, A., & Dewettinck, K. (2014). Polysaccharide-based oleogels prepared with an emulsion-templated approach. **ChemPhysChem**. 15(16), 3435-3439

Patel, A.R., Cludts, N., **Bin Sintang, M.D.,** Lesaffer, A., & Dewettinck, K. (2014). Edible oleogels based on water soluble food polymers: preparation, characterization and potential application. **Food & Function**. 5(11), 2833-2841

Poster presentation at international conference

September 2014 EuroFed Lipid Conference, Montpellier, France

Oral presentations at international conferences

September 2015 EuroFed Lipid Conference, Florence, Italy

May 2016 AOCS Annual Meeting, Salt Lake City, UTAH, USA